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ESTROGENS AND ADRENOCORTICAL FUNCTION IN MAN

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ABSTRACT

The administration of the synthetic estrogen, ethinyl estradiol, in an oral dosage of 0.5 mg. daily to normal male and female subjects produced a prompt elevation in the levels of plasma phenylhydrazine-reacting (Porter-Silber) chromogens and corticosterone. More than 80 per cent of the plasma phenylhydrazine-reacting chromogens could be accounted for as cortisol. The urinary corticoids were decreased to 30 to 50 per cent of the control levels after two to three weeks of estrogen administration. The urinary 17-ketosteroids and aldosterone showed little or no change. Cortisol and corticosterone when infused in 100 to 200-mg. quantities disappeared from the plasma at a decreased rate after administration of estrogen. Cortisone and the dihydro and tetrahydro metabolites of cortisol disappeared at a normal rate. Estrogen therapy was associated with a decrease in the rate of synthesis of cortisol and corticosterone. These markedly elevated levels of plasma cortisol after treatment with estrogen were not associated with the usual signs and symptoms of hypercortisolemia and this was interpreted in terms of an increase in the fraction of protein-bound cortisol, which is thought to be biologically inactive.

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IT HAS been known for many years that the female sex hormones and some of their synthetic substitutes produce adrenal hypertrophy (1) and a rapid loss of lipids from the adrenal cortex in rats (2, 3). These phenomena occurred only when the pituitary was present (4), and were reported to be associated with increased amounts of circulating corticotropin (5). Vogt (6, 7) and Holzbauer (8) showed in the rat, but not in the rabbit (6), that treatment with estrogen resulted in a decreased rate of secretion of corticosterone (the major adrenocortical steroid secretory product in the rat) in adrenal-vein blood collected under anesthesia. However, the corticosterone content of the adrenal glands (expressed as μg. per Gm. of tissue) of unstressed rats treated with estrogen was increased (8), and this was interpreted as a sign of increased resting secretion. The cholesterol concentrations in the serum, adrenal glands and livers of rats treated with estrogen was reduced (9), and the decreased adrenal-vein concentration of corticosterone was interpreted as the result of a decreased synthesis of cholesterol.

The administration of the synthetic estrogens, stilbestrol (10) and ethinyl estradiol (11), to males or to nonpregnant females produces an increase in the plasma levels of 17-hydroxycorticosteroids comparable to that which occurs during pregnancy (12). During pregnancy the increase in plasma steroid levels is most marked in the latter trimester at a time when there is also a marked increase in the elaboration of estrogen (13), and it is not associated with symptoms or signs of Cushing's syndrome such as may occur in association with either adrenocortical hyperplasia or neoplasia or administration of therapeutic doses of corticotropin or cortisone. Thus, in terms of adrenocortical function and cortisol metabolism, this elevated plasma cortisol level in the absence of symptoms and signs of hypercortisolemia have appeared to present a paradox. It therefore seemed of interest to evaluate the effect of estrogen on adrenocortical function and the plasma cortisol level, utilizing the more specific methods now available for assessing adrenocortical activity and steroid metabolism.

MATERIALS AND METHODS

Six normal subjects, 2 patients with active rheumatoid arthritis, 1 patient with cirrhosis, and 1 hypophysectomized patient served as the subjects of this study. The estrogen was administered as 0.5 mg. of ethynyl estradiol orally per day. The male subjects usually complained of slight breast tenderness, loss of libido, and, infrequently, of anorexia. Occasionally the female subjects complained of anorexia.

Plasma cortisol and urinary corticosteroids were determined by the modified (14) procedure of Silber and Porter (15) using phenylhydrazine and sulfuric acid. Urinary cortisol was determined by the double isotope derivative method (16). Urinary 17-ketosteroids were determined by the Zimmermann procedure, modified (17) from the Holtorff and Koch method (18). Plasma corticosterone was determined by the isotope

dilution method (19) or the double isotope dilution derivative assay. Urinary aldosterone was determined by the double isotope derivative method (20).

The steroids for intravenous administration were dissolved in ethanol and diluted to 300 ml. with 5 per cent dextrose in water to a final alcohol concentration of approximately 5 per cent. Following rapid infusion of the steroids, plasma samples were collected serially and assayed. Plasma steroid concentrations were plotted on semilogarithmic coordinates against time, and the rate of disappearance of the steroids from the plasma was expressed as a biologic half-time, *i.e.*, the time at which half of any given quantity remained in the plasma. Cortisol, dihydrocortisol and tetrahydrocortisol were assayed by the phenylhydrazine method. The specificity of this assay method was evaluated for each steroid[1] by isotope dilution (14), which indicated that 80 per cent or more of the steroid as measured by the phenylhydrazine assay represented the administered steroid. Cortisone and corticosterone were assayed by the isotope dilution method, since the phenylhydrazine assay for cortisone (21) and, to a lesser extent, the fluorometric assay for corticosterone (19) were not found to be specific for the determination of the steroids in plasma following their intravenous injection.

All analyses for carbon^{14} and tritium were carried out with the Packard liquid scintillation spectrometer using a toluene DPO (diphenyloxyzol), POPOP (phenyloxyzol-phenyloxyzolphenyl) phosphor. With a background of 100 c.p.m. this equipment yielded a maximum of 87 per cent efficiency for carbon^{14} and 23 per cent efficiency for tritium. Total urine radioactivity following the injection of cortisol-4-C^{14} was determined by counting a small aliquot of the urine (0.1 to 0.5 ml.) directly in the toluene phosphor containing added alcohol. A correction for loss of efficiency due to "quenching" was made by utilizing a double differential isotope dilution technique (22). The fractions of radioactive metabolites appearing as free and as glucuronide conjugates were determined as previously described (23), but with the use of the liquid scintillation spectrometer. The fraction of the injected tracer dose of cortisol-4-C^{14} excreted as cortisol-4-C^{14} was determined by reverse isotope dilution, using added carrier cortisol and paper chromatographic purification of the urine extract prior to phenylhydrazine assay.

The size of the rapidly exchangeable miscible pool of cortisol and the rate of turnover of the pool were determined by the previously described method (24). The pool size and rate of turnover of corticosterone were determined by a procedure similar to the one used for cortisol (25), except that tritium-labeled corticosterone (1.5 μc. per μg.) prepared by the Wilzbach technique (26) was used. The rates of turnover of cortisol and corticosterone were also determined by measuring the dilution of the isotope content of the injected labeled steroid and its metabolites by one or more of the urinary metabolites of the parent compound (25, 27).

Studies of the plasma protein binding of cortisol were carried out by use of the centrifuge-type apparatus and ultrafiltration procedures as described by Toribara (28). All of the subjects studied had zero plasma levels of cortisol as a result of suppression of

[1] Cortisol-4-C^{14} (1.47 mc./mM), cortisone-4-C^{14} (0.49 mc./mM) and corticosterone-4-C^{14} (1.47 mc./mM) were obtained from the Endocrine Study Section of the National Institutes of Health. C^{14}-labeled tetrahydrocortisol and dihydrocortisol were prepared enzymatically (42) from cortisol-4-C^{14} (1.47 mc./mM). Cortisol, 9α-fluoroscortisol, Δ^1-cortisol and corticosterone were labeled with tritium by the Wilzbach (24) technique. All of the labeled steroids were purified by paper chromatography to a constant specific activity. All of the radioactivity measurements were made with the Packard liquid scintillation spectrometer.

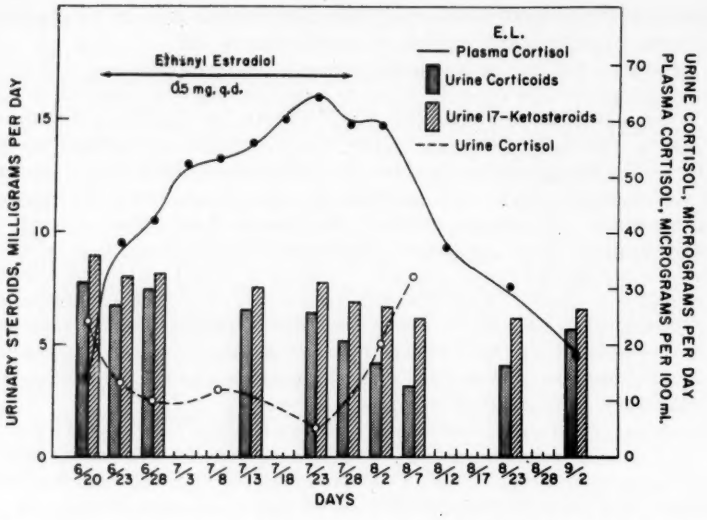

FIG. 1. Urinary steroid and plasma cortisol levels following administration of ethinyl estradiol to a normal female.

endogenous corticotropin secretion with small doses of Δ^1,9α-fluoro-16α-methylcortisol (Decadron). Heparinized plasma was mixed with cortisol-4-C^{14} and varying concentrations of carrier cortisol. Ultrafiltration was effected by centrifuging this solution, contained in Visking cellophane casing in Toribara tubes at approximately 740×g for twelve to sixteen hours at 37° C. (29). The tubes were filled with 5 per cent CO_2-95 per cent O_2 to keep the plasma buffered at physiologic pH. The cortisol was extracted from an aliquot of the starting mixture and the ultrafiltrate, with 10 volumes of dichloromethane. An aliquot of this solvent was dried in counting vials for radioactivity assay with the liquid scintillation spectrometer.

RESULTS

Figure 1 shows the plasma and urinary cortisol levels, and urinary corticosteroids and 17-ketosteroids in a normal young adult female (E.L.) after the oral administration of ethinyl estradiol (0.5 mg. per day) for a period of thirty-nine days. The plasma cortisol level was elevated above normal concentrations by the third day of treatment with estrogen and remained above normal for a month after the estrogen was discontinued. The 24-hour urinary 17-ketosteroids decreased only slightly from a control level of 8.9 mg. to a low of 6.8 mg. The 24-hour urinary corticosteroids declined moderately from a control level of 7.7 mg. to a low of 3.1 mg. on the tenth day after stopping estrogen. Urinary cortisol concentrations decreased rapidly from the control level of 25 μg. per twenty-four hours to a low of 4 μg. Total blood eosinophil counts at 7 a.m. were obtained periodically

throughout this study, and at no time did the levels fall to less than 60 per cent of the control value.

Table 1 shows the results of additional studies on the same woman (E.L.) and on 4 other normal subjects (2 men and 2 women) given estrogen. In all subjects receiving the estrogen for more than two weeks, there was a modest decline in urinary corticosteroid excretion. In 2 (E.L. and T.R.), urinary 17-ketosteroid excretion also decreased, although in E.L. the change was of questionable significance. In the 3 subjects in whom urinary aldosterone (free plus "acid-released") measurements were made, no significant decrease was noted. Urinary cortisol excretion in the 4 subjects studied fell rapidly to a subnormal level.

The plasma corticosterone level rose slightly in 2 of the 3 subjects studied. In all subjects the plasma cortisol level rose promptly. In Subject T.D. a significantly increased concentration of cortisol was noted by the second day of treatment and a marked increase to levels above normal by the third day. The concentration of plasma cortisol became stabilized at a level of 40 to 60 μg. per 100 ml., usually within two weeks after starting treatment. A further increase in the dosage of estrogen failed to produce an additional increase in the plasma cortisol value. In several instances the plasma cortisol concentration was also determined by the more specific isotope dilution method. In each instance the phenylhydrazine colorimetric assay proved to be approximately 80 per cent specific for cortisol.

Patient D.B. (Table 2) had active rheumatoid arthritis of two years' duration and had been maintained with 10–15 mg. of prednisone and 3.6 Gm. of aspirin daily for over a year. While he was in a state of only *partial* remission during therapy with 10 mg. of prednisone per day, ethinyl estradiol was started. During the preliminary control period, the level of plasma phenylhydrazine-reacting chromogens was 6–9 μg. per 100 ml. During administration of estrogen, the level rose to 67 μg. per 100 ml. Isotope dilution assays showed these plasma chromogens to consist entirely of prednisolone, both before and up to the fiftieth day of estrogen treatment. The fact that it was not possible to detect any cortisol in the plasma indicated complete suppression of adrenal synthesis of cortisol. Thirty-four days after starting the estrogen, the maintenance dosage of prednisone was decreased to 7.5 mg. per day, and shortly thereafter the patient had a mild symptomatic relapse; however, the plasma prednisolone level continued elevated. During the entire course of estrogen treatment, urinary corticosteroid excretion was below 3 mg. per twenty-four hours. Patient R.B. (Table 2) had moderately active rheumatoid arthritis of seven years' duration. After initiating treatment with estrogen, the plasma cortisol rose to levels of 56 μg. per 100 ml. and the 24-hour urinary excretion of corticosteroids declined to 5.5 mg. from a control value of 7.3 mg. During the thirty

TABLE 1. PLASMA AND URINARY STEROID LEVELS IN 5 NORMAL CONTROL SUBJECTS DURING ADMINISTRATION OF ETHINYL ESTRADIOL (0.5 MG. PER DAY, ORALLY)

Subject	Days from start of estrogen admin.	Plasma steroids			Urinary steroids			
		Cortisol (PNH)* (μg./100 ml.)	Cortisol (isotope dil. (μg./100 ml.)	Corticosterone (isotope dil.) (μg./100 ml.)	Corticoids (mg./24 hrs.)	17-KS (mg./24 hrs.)	Aldosterone (μg./24 hrs.)	Cortisol (μg./24 hrs.)
E.L., ♀		14	11	0.8	7.7	8.4	8	25
Normal	3	38	35	—	7.1	8.2	—	12
	9	42	—	—	7.5	8.2	—	10
	24	56	49	1.0	7.0	7.7	7	—
	34	63	—	—	6.9	8.0	—	4
	39	59	50	—	5.2	7.0	—	—
T.R., ♂		12	10	0.8	4.3	14.0	13	44
Normal	7	35	—	—	4.0	10.0	—	10
	16	39	—	—	2.3	6.6	—	10
	30	46	44	—	2.3	6.5	—	—
	60	52	47	2.2	1.9	6.6	8	7.7
D.M., ♀		17	16	0.8	7.4	8.2	—	17
Normal	7	35	—	—	7.8	8.4	—	3.0
	14	46	—	—	4.2	8.3	—	4.6
	21	47	44	1.8	3.5	8.7	—	4.8
T.D., ♂		14	11					
Normal	1	19	16					
	2	26	23					
	3	30	26					
	5	44	40					
	7	49	47					
	14	56	50					
	18	60	50					
D.S., ♀		9			8.1	11	8	37
Normal	14	40			7.1	12	8	3.6

* PNH = Porter-Silber chromogens.

days of treatment with ethinyl estradiol he showed no subjective or objective improvement in the symptoms and signs of arthritis. Twenty-four days after estrogen was discontinued, the plasma cortisol level had returned to normal. Oral cortisol was then started in sufficient dosage to elevate the

TABLE 2. PLASMA AND URINARY STEROIDS IN 2 PATIENTS WITH RHEUMATOID ARTHRITIS TREATED WITH ETHINYL ESTRADIOL

Patient D.B., ♂		Patient R.B., ♂		
Day from start of estrogen admin.	Plasma prednisolone (μg./100 ml.)	Days after estrogen admin.	Plasma cortisol (μg./100 ml.)	Urinary corticoids (mg./day)
Control* (May 15)	6	Control (June 6)	12	7.3
Control* (May 29)	8			
Control* (June 8)	9	*Estrogen*		
		3*	19	6.4
Estrogen		6	25	5.5
5	18	11**	33	4.9
15	24	19	49	4.0
22	40	26	46	5.0
29	39	40†	56	5.5
36†	54			
43	64	47	32	
50	67	54	22	
		64	16	
		Cortisol		
		68‡	16	
		70§	—	
		71	25	
		74‖	22	
		80	32	

Patient D.B.—* Prednisone, 10 mg./day.
† Prednisone reduced to 7.5 mg./day.
Patient R.B.—* Norethindrone, 160 mg./day. Subsequently it was found to be contaminated with estrogen.
** Ethinyl estradiol started. Norethindrone discontinued.
† Ethinyl estradiol stopped.
‡ Cortisol, 40 mg./day.
§ Cortisol, 100 mg./day.
‖ Cortisol, 60 mg./day.

plasma cortisol level to 25 μg. per 100 ml., and at this time there was definite relief of symptoms and objective signs of improvement.

Figure 2 shows the plasma cortisol response to intravenous corticotropin in 2 normal subjects treated with estrogen. In both, the concentration of cortisol rose to levels far in excess of those seen in normal subjects given corticotropin. The plasma level rose promptly from an initial high value of about 60 μg. per 100 ml. to more than 135 μg. per 100 ml. at twelve and at twenty-four hours. Both of these subjects experienced the usual "flushed"

response within twenty-four hours after the start of the infusion. No eosinophils were found in the blood of either subject at twenty-four hours. In both, the plasma cortisol level determined by isotope dilution approximated that found by the phenylhydrazine method.

The administration of estrogen markedly slowed the rate of disappear-

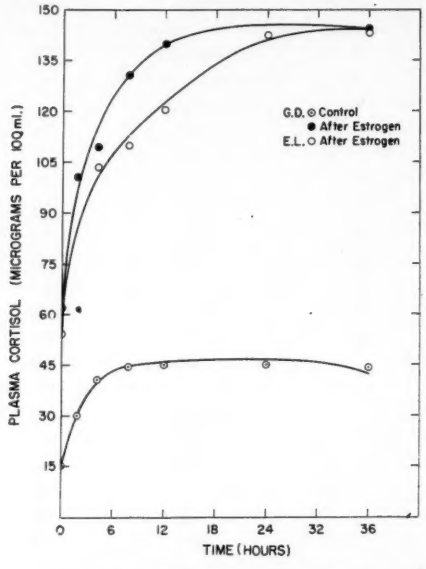

FIG. 2. Plasma cortisol levels in estrogen-treated normal subjects (G.D., ♂; E.L, ♀) following infusion of corticotropin.

ance of infused cortisol from the plasma (Table 3). In Subject T.R., on day 84 of estrogen treatment, the plasma cortisol concentrations after infusion of cortisol were determined by both the phenylhydrazine and the more specific isotope dilution assay; the resulting biologic half-times were identical. Thus it would appear that the prolonged half-time of cortisol after administration of estrogen is an index of a decreased rate of metabolism of cortisol and not a reflection of a delayed disappearance of plasma phenylhydrazine-reacting cortisol metabolites. The metabolites of cortisol (dihydrocortisol and tetrahydrocortisol) were metabolized at a rate close to normal; however, in Subject E.L. there was a slightly slower metabolism of both these steroids. Also, in this same patient, the synthetic steroids Δ^1-cortisol, 9α-fluorocortisol, and Δ^1,9 α-fluorocortisol were cleared from the plasma at a rate slower than normal. Cortisone metabolism did not appear

to be influenced by the prior estrogen therapy; however, the metabolism of infused corticosterone was slightly slowed.

In Subject T.R., a man treated with estrogen for ten days (days 90 to 100 following initiation of the first 84-day course of treatment), the plasma cortisol level was reduced to zero following administration of 2 mg. of

TABLE 3. EFFECT OF ESTROGEN ON BIOLOGIC HALF-TIMES OF INFUSED STEROIDS

Subject	Day from start of estrogen admin.	Biologic half-time (hrs.) for				
		F*	B	E	DHF	THF
E.L., ♀	control	1.8	1.3	0.5	0.8	0.8
Normal	14	2.6	—	—	—	—
	15	—	2.1	—	—	—
	25	—	—	—	1.1	—
	26	—	—	—	—	1.2
	28	3.3	—	—	—	—
T.R., ♂	control	1.8	1.2	0.6	0.6	0.8
Normal	21	—	1.8	—	—	—
	28	3.3	—	—	—	—
	44	—	—	—	0.8	—
	45	—	—	—	—	0.8
	50	—	2.3	—	—	—
	56	—	—	0.6	—	—
	84	4.4	—	—	—	—
T.D., ♂	control	1.6	1.4	—	0.6	0.5
Normal	14	2.2	—	—	—	—
	15	—	—	—	0.5	—
	16	—	—	—	—	0.6
	18	—	1.6			
	21	2.8				

* F = cortisol; B = corticosterone; E = cortisone; DHF = dihydrocortisol; THF = tetrahydrocortisol.

Δ^1,9α-fluoro-16α-methylcortisol. Plasma from this subject was used to determine the fraction of bound and unbound cortisol at different plasma cortisol concentrations. Figure 3 shows the results of these studies in Subject T.R. and in 3 normal adult females after suppression of adrenal steroid secretion. In the 3 normal women the ratio of total to unbound cortisol was constant up to a plasma cortisol concentration by about 20 μg. per 100 ml. However, above 40 μg. per 100 ml. the curve became linear up to at least 100 μg. per 100 ml., with a lower ratio of total to unbound cortisol. In the

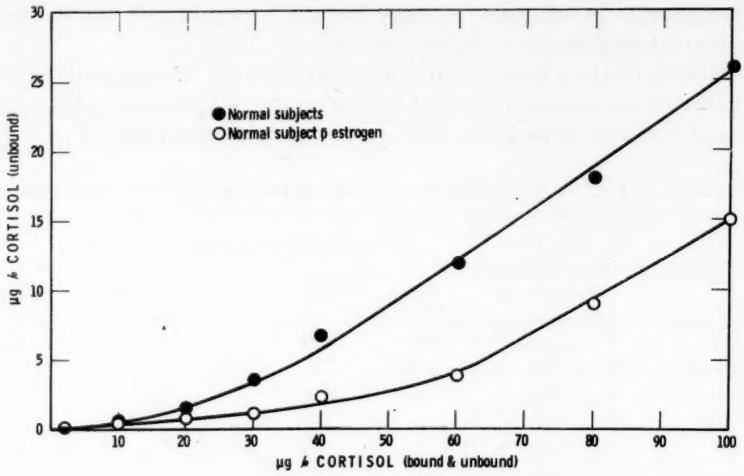

FIG. 3. Fraction of bound and unbound cortisol at various plasma cortisol concentrations. The data on untreated normal subjects represent the mean values obtained on 3 normal adult females. The other curve represents data on an estrogen-treated normal male (Subject T.R.).

male subject treated with estrogen the curves were also linear, but with a distribution coefficient for cortisol more in favor of plasma proteins. In both groups the data suggest that there are at least two components in plasma that bind cortisol. One of these has a high affinity for the hormone but is saturated at low concentrations, whereas the other has a lower affinity but a larger capacity.

Table 4 shows the percentage of infused cortisol excreted in the urine following the infusion of C^{14}-labeled and unlabeled cortisol into 2 normal subjects, before and after treatment with estrogen. After infusion of a tracer dose of labeled cortisol, estrogen produced a slight decrease in the fraction excreted as cortisol. After infusion of pharmacologic amounts of unlabeled cortisol, the values for urinary free cortisol (though much higher in the control period) decreased after estrogen therapy. There were no significant differences in the fraction of administered cortisol excreted as phenylhydrazine-reacting steroid metabolites conjugated with glucuronic acid. After injection of cortisol-4-C^{14} there was no significant difference in the total quantity of radiometabolites appearing in the 24-hour urine. The data, however, do not furnish any information regarding the rate of appearance of these urinary metabolites.

Table 5 shows the effects of administered estrogen on the miscible pool and rate of turnover of cortisol in 3 normal subjects. In 2 of them (T.R.

TABLE 4. EFFECT OF ESTROGEN ON 24-HOUR URINARY CORTISOL AND METABOLITES OF CORTISOL AFTER INTRAVENOUS INFUSION OF LABELED AND UNLABELED CORTISOL

Subject	Day from start of estrogen admin.	0.2 mg. Cortisol-C^{14} i.v.				200 mg. Cortisol i.v.	
		% excreted (total)	% total excreted as			cortisol (%)	glucuronides* (%)
			free	cortisol	glucuronides*		
E.L., ♀	control	79	3.0	0.1	48	2.8	20
Normal	40	74	3.5	0.05	40	1.5	20
T.R., ♂	control	75	5.0	0.05	55	2.5	28
Normal	30	76	8.0	0.02	48	1.3	22

* Phenylhydrazine-reacting steroids released by β-glucuronidase.

and E.L.), estrogen increased the size of the miscible pool. Calculated on the basis of the fraction of the pool replaced per hour, there was a decreased rate of turnover after administration of estrogen. Moreover, when the rate of turnover was determined by the change in specific activity of one of the

TABLE 5. EFFECT OF ESTROGEN ON MISCIBLE POOL AND TURNOVER RATE OF CORTISOL

Subject	Day from start of estrogen admin.	Pool (mg.)	$t_{\frac{1}{2}}$* (hrs.)	k**	Turnover	
					mg./hr.†	mg./day‡
T.R., ♂	control	2.2	1.8	0.38	0.83	12.5
Normal	30	3.1	4.7	0.15	0.46	6.8
E.L., ♀	control	1.2	1.1	0.64	0.77	12.0
Normal	40§	3.9	6.9	0.10	0.39	5.5
	52	2.9	3.5	0.20	0.58	8.0
G.D., ♂	control (ACTH)‖	9.7	0.9	0.77	7.5	200.0
Normal	15 (ACTH)‖	21	2.0	0.35	7.4	190.0

* Time for specific activity of cortisol to be reduced by half.

** Fraction of pool replaced per hour.

† Calculated by the kinetic method of assay of change in specific activity of plasma cortisol after injection of 200 μg. of cortisol-4-C^{14}.

‡ Calculated from specific activity of tetrahydrocortisone in 24-hour urine collected after 200 μg. of cortisol-4-C^{14}.

§ Estrogen stopped on day 40.

‖ Continuous intravenous infusion of corticotropin (4 U./hr.) for 36 hours. Cortisol-4-C^{14} injected at twelfth hour.

TABLE 6. EFFECT OF ESTROGEN ON MISCIBLE POOL AND TURNOVER RATE OF CORTICOSTERONE

Subject	Days from start of estrogen admin.	Pool (mg.)	$t_{\frac{1}{2}}$* (hrs.)	k**	Turnover	
					mg./hr.†	mg./day‡
T.R., ♂	control	0.30	1.0	0.70	0.21	2.3
Normal	40	0.60	2.1	0.33	0.20	1.4
D.S., ♀	control	0.20	0.8	0.85	0.17	2.0
Normal	14	0.25	1.6	0.43	0.11	1.4

* Time for specific activity of corticosterone to be reduced by half.

** Fraction of pool replaced per hour.

† Calculated by the kinetic method of assay of change in specific activity of plasma corticosterone after injection of 7 μg. of corticosterone-H^3.

‡ Calculated from specific activity of tetrahydrocorticosterone in 24-hour urine collected after injection of 7 μg. of corticosterone-H^3.

urinary metabolites of cortisol (tetrahydrocortisone), the daily rate of turnover of cortisol was decreased. In Subject E.L., specific activity assays of urinary tetrahydrocortisol also showed a change comparable to the change in specific activity of tetrahydrocortisone.

In normal Subject G.D. the pool and rate of turnover of cortisol were determined after treatment with corticotropin, and after fifteen days of treatment with estrogen. After estrogen therapy the pool of cortisol was increased, but not the fractional rate of turnover.

Table 6 shows the data on the miscible pool and rate of turnover of corticosterone in 2 normal subjects, both before and after treatment with estrogen. It is apparent that the effects of the estrogen on corticosterone metabolism were less striking than the effects on cortisol metabolism. In Subject T.R., specific activity assays of urinary tetrahydro-11-dehydrocorticosterone also showed changes similar to those for tetrahydrocorticosterone.

DISCUSSION

Following administration of estrogen the level of plasma phenylhydrazine-reacting chromogens was elevated. This confirms the previously published data (10, 11, 30); however, it seemed necessary to prove that the three- to fourfold increase in the level of these chromogens represented predominantly plasma cortisol. It was conceivable that the chromogens represented other 17-hydroxycorticosteroids, or perhaps nonsteroidal substances. In the several instances in which the plasma was assayed by both the modified Silber-Porter method and the more selective isotope dilution

technique, a good correspondence was noted. The isotope dilution assay usually yielded slightly lower values for plasma cortisol; however, in all instances, 80 per cent or more of the phenylhydrazine-reacting chromogen was cortisol.

The general reliability of the Silber-Porter procedure as an index of elevated plasma cortisol levels after estrogen administration was supported by the following additional observations: 1) In Subject T.R. during estrogen therapy Δ^1,9α-fluoro-16α-methylcortisol in small doses decreased the levels of plasma cortisol and phenylhydrazine-reacting chromogens to zero. 2) In 1 hypophysectomized patient, and in 1 patient with rheumatoid arthritis maintained with prednisone, estrogen therapy did not cause any elevation of plasma cortisol concentration. 3) In Subject T.R., the intravenous infusion of 0.5 mg. of ethinyl estradiol over a period of six hours did not produce an increase in the plasma level of phenylhydrazine-reacting chromogens or of cortisol. 4) The plasma cortisol level remained elevated above normal for two to three weeks after discontinuance of estrogen therapy. These data demonstrate that the administered estrogen did not contribute directly to any of the chromogens measured.

The very high plasma levels of cortisol appear to present a paradox since it has been presumed that plasma cortisol concentration is maintained in the normal range by a negative feedback control of the adrenocortical secretion rate in such a manner that changes in the rate of secretion are brought about by opposite changes in the cortisol concentration of the plasma (31). The normal plasma cortisol levels in thyroid and liver disease in the presence of different rates of synthesis have been interpreted in terms of this negative feedback control via the anterior pituitary (32, 33). Thus an elevated concentration of plasma cortisol following estrogen therapy should inhibit the release of corticotropin by the pituitary, and as a consequence cause a decreased rate of production of cortisol, thereby reestablishing the normal level of plasma cortisol.

Even though these plasma cortisol concentrations were comparable to those seen after intravenous injection of corticotropin or the administration of 100–200 mg. of cortisone per day, there was no significant decrease in blood eosinophils. Also, in a patient with active rheumatoid arthritis known to show a good response when the plasma cortisol level was 25 μg. per 100 ml. after oral cortisol, there was no such favorable response after estrogen therapy when the plasma cortisol level was 50 μg. per 100 ml. Another patient with rheumatoid arthritis who gave a history of repeated relapses when the prednisone dosage was reduced to less than 10–15 mg. per day (plasma prednisolone level 6 μg. per 100 ml.) showed a mild exacerbation during estrogen therapy when the dosage of prednisone was reduced, although the plasma prednisolone level remained as high as 60 μg. per 100

ml. Thus, it would appear that the plasma concentration of cortisol as determined chemically may not always be a reliable index of the amount of biologically active hormone in the extracellular fluid, or at its locus of action.

It is known that cortisol, corticosterone and aldosterone exist in the blood in two different physiochemical states, namely, nonprotein bound and protein bound (34–39). Conventional chemical methods measure both the bound and unbound. The equilibrium between bound and unbound cortisol will affect the distribution (vascular-extracellular-intracellular), transport, excretion and biotransformation of the steroid, and indirectly via the negative feedback mechanism influence the rate of synthesis of cortisol. The negative feedback mechanism would most likely be responsive only to the unbound (or biologically active) cortisol.

In the normal subjects treated with estrogen, the elevated plasma cortisol (and corticosterone) concentration was not associated with any of the signs or symptoms of hyperadrenocorticism; this indicates that the protein-bound cortisol is probably biologically inactive.

In the estrogen-treated normal subjects the concentration of unbound cortisol was only 2 μg. per 100 ml. at a level of approximately 40 μg. per 100 ml. of total plasma cortisol, whereas in the normal untreated subjects the concentration of unbound cortisol was approximately 7 μg. per 100 ml. at a comparable level of plasma cortisol. Recently Sandberg and Slaunwhite (40) also reported that the administration of estrogen to normal subjects causes a great increase in plasma transcortin binding and the binding capacity of cortisol-4-C^{14}.

The absence of signs and symptoms of hyperadrenocorticism might have been interpreted as the result of very high cortisol levels at 8–9 a.m. with very low or zero levels throughout most of the remainder of the day. In both Subjects E.L. and T.R., in a few instances plasma cortisol was measured late in the afternoon or evening, and the concentration was usually lower than in the morning but higher than normal (>20 μg. per 100 ml.). It has recently been reported that in patients treated with estrogens the diurnal variation in plasma cortisol concentration is maintained (30).

The plasma protein (or proteins) that binds and transports the steroids may also protect them from excessively rapid transformation by hepatic enzymes, and thus influence the rate at which they are metabolized. When infused rapidly in quantities in the range of 100 to 200 mg., most of the adrenal steroids disappear from the plasma at rates approximately proportional to their concentration in peripheral blood plasma (23, 27). If the steroids are distributed within the plasma, extracellular space and tissues, the plasma concentration may be expressed as a simple exponential function of time, and the rate of metabolism may thus be expressed as a "biologic half-time."

In the normal subjects treated with estrogen, the infused cortisol and corticosterone and certain of the synthetic steroids disappeared from the plasma at a slower rate. The biologic half-time for cortisol-4-C^{14} administered in tracer quantities was shorter than the half-time observed in most of these subjects following the larger doses of unlabeled cortisol. This difference in rate of disappearance of tracer and pharmacologic doses of cortisol has also been observed in normal untreated subjects (27, 33), and has been interpreted as due to differences in the fraction of unbound and protein-bound steroid at physiologic (tracer) and pharmacologic (therapeutic) levels. The differences observed between the normal subjects before and after estrogen therapy may be similarly explained.

The increase in plasma protein binding and in the binding capacity of cortisol (and corticosterone) may represent only one factor influencing the rates of metabolism. It would not seem reasonable to postulate a competitive inhibition between cortisol and the estrogens at an enzymatic level, since the defect in biotransformation persists for weeks after the estrogen is discontinued. The main site of corticosteroid inactivation is in the liver, and it may be that estrogen therapy in some manner alters the level of one of the co-enzymes (TPNH) or the level of the TPNH-linked steroid dehydrogenases (the soluble 5-β, or C-20 or 5-α particulate enzymes) in the liver necessary for the enzymatic inactivation of the steroids. The A-ring reduced C-3 ketone and C-20 reduced steroids appear to represent the predominant initial steps in the degradation of the steroids and account for approximately half of the metabolites of cortisol appearing in the urine (41).

The rate of metabolism of cortisone was not altered by estrogen therapy; however, the predominant initial reaction in its metabolism, though dependent on TPNH, appears to be a reduction of the C-11 ketone to an 11-hydroxyl function (cortisol). Also, the rate of metabolism of dihydrocortisol was not altered, but again the pyridine-nucleotide dependent (TPNH or DPNH) steroid dehydrogenase involved in reduction of the C-3 ketone group is a distinctively different enzyme (42). The metabolism of tetrahydrocortisol, except in Subject E.L., was not significantly altered by administered estrogen. This would indicate that there was no impaired capacity for conjugation of the A-ring reduced steroid metabolites. Also, the appearance of a normal fraction of the phenylhydrazine-reacting steroid glucuronides in the urine after infusion of cortisol indicates no significant alteration in the capacity of the liver to conjugate the steroid metabolites (tetrahydrocortisone, tetrahydrocortisol, allotetrahydrocortisol) with glucuronic acid.

In patients with cirrhosis and myxedema (32, 33) the negative feedback mechanism operates so efficiently that delayed hepatic inactivation is not associated with a plasma cortisol level above approximately 20 μg. per 100

ml. However, in estrogen-treated subjects, with the increased protein binding of cortisol, the plasma cortisol level rises due to its slower rate of inactivation by the liver. Under these circumstances the concentration of unbound cortisol does not become elevated sufficiently to act as an inhibitor of corticotropin secretion until the total plasma cortisol level is at least twice normal (40 μg. per 100 ml).

A partial suppression of adrenocortical function does appear to occur when the total plasma cortisol becomes elevated sufficiently to produce an increased concentration of unbound cortisol. The studies with cortisol-4-C^{14} and corticosterone-H^3 showed that the fractional rate of steroid turnover was reduced after administration of estrogen. This was associated with an increase in the size of the miscible pool, comparable to the increases observed in the plasma steroid levels. The net effect of these two changes was such that the rate of synthesis, expressed as mg. per hour, was significantly reduced. The turnover rates, based on the dilution of one or more of the urinary metabolites of cortisol or corticosterone, also confirmed the decrease in synthesis of these two steroids.

In the rat, corticosterone synthesis as measured by the more direct method of adrenal-vein accumulation is also decreased after administration of estrogen (7).

Urinary and biliary losses of cortisol and corticosterone are negligible (23), and steroid protein binding may protect the hormone from being filtered through the renal glomeruli. Normally only a fraction of the cortisol produced each day is lost by way of the kidneys (20 to 150 μg. per day (43)). In the subjects treated with estrogen, urinary cortisol decreased. This might not have been anticipated, for even though the plasma level of bound cortisol was increased, there may have been a larger quantity of unbound cortisol at a total plasma cortisol level of 40 to 50 μg. per 100 ml. Thus the decreased urinary excretion of cortisol may represent either a failure in the precision of measurement of the quantity of unbound cortisol, or an increase in renal tubular reabsorption. Filtered cortisol has been reported to be reabsorbed to the extent of 80 to 90 per cent normally (44, 45).

In all of the subjects studied the urinary corticoids decreased after estrogen therapy. Even though urinary corticoid assays measure only 20 to 40 per cent of the metabolites of cortisol, a decreased concentration usually indicates decreased synthesis of cortisol (27). This is especially so if it can be shown that administration of estrogen caused no alteration in the pattern of metabolites. The studies in the 2 subjects given labeled and unlabeled cortisol demonstrated that there was no marked change in the fractions of the metabolites excreted as glucuronide and free, or in the phenylhydrazine-reacting steroid metabolites conjugated as glucuronides. The changes in urinary 17-ketosteroids were minimal when compared to those

in corticoids. However, this assay represents primarily a measure of the capacity of the adrenal cortex to secrete C-19 androgenic steroids rather than cortisol or corticosterone. In the few subjects studied, no significant change in aldosterone excretion was noted. However, this assay measures such a small fraction of the secreted aldosterone (<5 per cent; unpublished observations) that any interpretations concerning aldosterone secretion rates are tenuous at best. The mechanisms for control of aldosterone secretion are entirely different from those regulating cortisol and corticosterone secretion, so that it might not be expected that changes in aldosterone secretion would parallel changes in the secretion of cortisol and corticosterone.

The extremely high plasma cortisol levels (140 μg. per 100 ml.) in the estrogen-treated normal subjects given intravenous corticotropin indicated that the adrenal cortex was responding in a normal way to corticotropin, and prior to corticotropin stimulation was not secreting cortisol at a maximal rate. These high plasma cortisol levels were at least three times the levels (40–50 μg. per 100 ml.) obtained in normal subjects after a similar infusion of corticotropin. Thus, in the estrogen-treated subjects, these elevated concentrations of cortisol must have represented either a hyperresponse of the adrenal cortex to corticotropin, or a delayed metabolism of the excess steroid. The latter is undoubtedly the correct explanation, since it was shown that estrogen actually depressed adrenocortical secretion, and in Subject G.D. the rate of turnover of cortisol under the influence of optimal stimulation with corticotropin was no different from the rate of secretion prior to estrogen therapy, although the plasma cortisol level was three times higher. During administration of estrogen, the fractional rate of turnover of the cortisol pool was much decreased. In both subjects given corticotropin (E.L. and G.D.) while receiving estrogens, the high plasma cortisol levels, as expected, produced an eosinopenia.

It is interesting that many of these changes in cortisol metabolism during estrogen therapy are similar to those seen during the latter trimester of pregnancy, namely, increased concentration of plasma cortisol (12, 46), increased cortisol-protein binding (38, 40), delayed rate of metabolism of infused cortisol (47, 48), and normal or decreased urinary excretion of corticoids (49, 50, 51). The pregnant state is not associated with the symptomatic stigmata of Cushing's syndrome, even though plasma cortisol levels are comparable to those usually found in classic Cushing's syndrome. Plasma estrogen levels are also markedly elevated during the last trimester of pregnancy (13).

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THE EFFECT OF ESTROGEN ADMINISTRATION ON THE METABOLISM AND PROTEIN BINDING OF HYDROCORTISONE

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ABSTRACT

A study has been made of the effect of estrogen administration on the plasma hydrocortisone level in normal subjects, with and without concomitant cortisone therapy. Plasma hydrocortisone levels in some instances fall progressively during the control period with cortisone alone. This is reversed by administration of estrogen. By use of ultrafiltration at 37°C. and double isotope tracer techniques the protein-bound and nonprotein-bound fractions of plasma hydrocortisone were studied. Approximately 5–10 per cent was not protein bound. This percentage was reduced, but absolute values remained the same, when estrogen was given. The rise in the plasma level of hydrocortisone represented an increase in the protein-bound fraction, and *in vitro* techniques revealed an increase in the binding protein, or in the number of sites. The increased plasma levels due to estrogen therapy were not associated with depression of eosinophils, which suggests that only the nonprotein-bound fraction is biologically active. The half-time for disappearance of plasma hydrocortisone is considerably prolonged by administration of estrogen; this probably represents protection from destruction by the liver by means of greater protein binding. The effects of estrogen in altering hydrocortisone metabolism are comparable to those produced by pregnancy.

ADMINISTRATION of estrogen to human subjects has been reported to lower the urinary excretion of metabolites of hydrocortisone (1), decrease the excretion of administered cortisone (2), increase the concentration of plasma 17-hydroxycorticosteroids in patients with normal adrenal

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function or Addison's disease (3), and cause an exaggerated response of the plasma 17-hydroxycorticosteroids when ACTH is given simultaneously (4). In guinea pigs it decreases the response of the urinary ketogenic steroids to corticotropin (5). The changes in plasma and urinary corticoids resemble those found during pregnancy, and Martin and Mills (6) have suggested that the changes they found in hydrocortisone metabolism during pregnancy might be the result of the high production of estrogen. Gemzell (7) postulated that the high plasma levels of 17-hydroxycorticoids which he observed in pregnant patients might be the result of stimulation of the pituitary-adrenal system. However, the absence of comparable increases in urinary excretion of corticoids (6, 8) is against this interpretation. Furthermore, Migeon *et al.* (9) showed that there is a decreased rate of removal of hydrocortisone from the plasma in late pregnancy. This has been confirmed by Christy *et al.* (10).

The studies reported here were designed to follow the effect of estrogen therapy on plasma and urinary corticoids during cortisone administration, and to separate plasma hydrocortisone into protein-bound and nonprotein-bound fractions. These fractions were determined during treatment with estrogen, when no exogenous corticoids were given. The rate of removal of C^{14}-hydrocortisone from the plasma was measured prior to and during administration of estrogen.

MATERIAL AND METHODS

Subjects. Three normal young adults (2 male, 1 female) and 1 patient (male) with cirrhosis of the liver were given 100 mg. of cortisone acetate intramuscularly once daily for two weeks. Samples of plasma were drawn daily at 8 a.m. prior to the injection of cortisone, and 24-hour collections of urine were made. After five to ten control days, estradiol benzoate was given in a dosage of 2 mg. once a day intramuscularly while cortisone was continued.

Five normal adults (4 female, 1 male) had control plasma samples drawn at 8 a.m. for measurement of total and ultrafiltrable hydrocortisone. After five days, 1.0 mg. of ethinyl estradiol was given daily orally for seven days to the 4 women, and 2 mg. of estradiol benzoate once a day intramuscularly to the man. Circulating eosinophils were measured daily during the whole period of study.

17-Hydroxycorticosteroids (17-OH-CS). Plasma 17-OH-CS levels were determined by the technique of Peterson *et al.* (11). Urinary 17-OH-CS excretion was measured by a technique similar to that used for plasma, but following glucuronidase hydrolysis.

Total and nonprotein-bound plasma hydrocortisone. Ultrafiltrate of plasma was obtained using the apparatus of Toribara (12). A 15-ml. sample of plasma was placed in soaked Visking tubing, inserted into the apparatus, gassed with 5 per cent carbon dioxide in oxygen, and centrifuged overnight in a temperature-controlled centrifuge at 37° C. It is essential to control the temperature because binding is greatly increased at lower temperatures (13). Determination of total hydrocortisone was made on 2 ml. of plasma, and of the nonprotein-bound steroid on 5 to 6 ml. of ultrafiltrate. C^{14}-labeled hydrocortisone was added to the sample in amounts up to 0.12 μg. prior to extraction with 6 volumes

of dichloromethane. The extract was washed with $N/5$ sodium carbonate and water and chromatographed on the system toluene:methanol:water (10:7:3). The hydrocortisone zone was eluted with ethanol, dried, and acetylated with tritium-labeled acetic anhydride in pyridine. The acetate was chromatographed on the systems cyclohexane:dioxane: methanol:water (14:4:2:1) and cyclohexane:benzene:methanol:water (5:10:10:2). It was then oxidized with 0.2 per cent potassium chromate in glacial acetic acid for exactly five minutes, and the cortisone acetate run in the system carbon tetrachloride: methanol:water (4:4:1). The cortisone acetate region was eluted with ethanol and counted for C^{14} and tritium in a liquid scintillation spectrometer. The specific activity of each batch of acetic anhydride was checked by acetylating pure steroids and measuring the ultraviolet absorption of the chromatographed steroid acetate.

Rate of removal of plasma hydrocortisone. This was assessed by infusing 2 to 4 μc. of C^{14}-hydrocortisone intravenously in 20 ml. of physiologic sodium chloride solution and taking 5 samples of blood at forty-minute intervals. Tracer amounts of C^{14}-labeled cortisone were added to each sample of plasma. Each sample was extracted with 6 volumes of dichloromethane, washed with $N/5$ sodium carbonate and water and the extract chromatographed in the system hexane:acetone:water (4:2:1). The zone containing cortisone and hydrocortisone (which separate very little in three hours) was eluted and run in the Bush C system. The zones of radioactivity from the 2 steroids are sharply separated by this technique and there is no appreciable interference one with the other. The strips were developed with blue tetrazolium in sodium hydroxide (20 mg. per 100 ml.) and heated in an oven at 80° C. to develop the soda fluorescence. This was measured on the paper in a fluorimeter of the type described by Ayres *et al.* (14). The radioactivity in both the cortisone and hydrocortisone zones was then measured by placing the appropriate zone of the strip in the liquid used in the liquid scintillation counter. The efficiency of this method of counting is approximately 20 per cent. The cortisone count was compared with that of a standard lane containing the amount of C^{14}-cortisone added to the original plasma sample. In this way correction was made for losses throughout the procedure.

The corrected value for C^{14}-hydrocortisone, as c.p.m. per 100 ml. of plasma, was plotted on semilog paper against time. From this graph was read off the value for $t_d^{1/2}$, the half-time for disappearance, or the time taken for the plasma radioactivity to be reduced by half. This method was found to be more satisfactory than working with specific activities, because with the larger amounts of C^{14}-hydrocortisone there was partial suppression of endogenous corticotropin and consequently a delay in the fall of specific activity.

In vitro *determination of protein binding of hydrocortisone.* Endogenous hydrocortisone production was suppressed by administration of 2 mg. of 9α-fluoro-16α-methylprednisolone (dexamethasone) eight hours prior to withdrawal of blood. To separate aliquots of plasma, increasing amounts of hydrocortisone were added together with C^{14}-labeled steroid. After extraction of a portion of the plasma for measuring total radioactivity, each aliquot was ultrafiltered and the ultrafiltrate extracted and counted for C^{14}. The percentage of steroid which was ultrafiltrable was plotted against the concentration of hydrocortisone in the plasma.

RESULTS

Demonstration of hydrocortisone in ultrafiltrate of plasma

A heparinized pool of plasma was ultrafiltered in aliquots until 75 ml. of ultrafiltrate was obtained. C^{14}-cortisone was added, and then the whole was

extracted with 6 volumes of dichloromethane and chromatographed on the system hexane, acetone and water and the Bush C system. The presence of hydrocortisone was demonstrated by the Rf values on the two systems, the reduction of blue tetrazolium and the amount of soda fluorescence. Quantitation was by means of soda fluorescence. Correction for losses was made by counting the C^{14}-cortisone on the Bush C chromatogram.

Concentration of ultrafiltrable hydrocortisone	= 1.0 μg. per 100 ml.
Concentration as estimated by double tracer technique	= 0.94 μg. per 100 ml.
Concentration of total plasma hydrocortisone in pool, by chromatography and soda fluorescence	= 10 μg. per 100 ml.
Proportion as ultrafiltrable hydrocortisone	= 10% of total.

Response to estrogen during cortisone therapy

The results in all 4 subjects are shown in Figure 1. The fall in plasma hydrocortisone concentration in some persons during cortisone therapy has previously been reported (15). In the present series, the reduction in plasma 17-OH-CS concentration was most striking in normal Subjects 1 and 4 (Fig. 1). The rise in the plasma values during estrogen therapy was most striking in those in whom the values had fallen during the control period. In Subject 3 (cirrhosis), the rise in the level of plasma 17-OH-CS was dramatic, but in the absence of confirmatory evidence, it would be unwise to accept this value as hydrocortisone. The urinary excretion of intramuscularly administered corticosteroid was low; this has been previously observed by Prunty (16). In all 4 subjects there was a tendency for the urinary excretion to rise during the control period. In Subject 1 the striking rise in the plasma 17-OH-CS level during estrogen therapy was associated with a fall in the urinary excretion.

In all cases there was a delay before the plasma level began to rise. This suggests that the estradiol did not contribute to the Porter-Silber chromogen, a point which has also been directly tested by others (3, 4). The work reported here involved chromatography of the plasma steroids and confirms the observation that there is a rise in the level of hydrocortisone as such. The dose of cortisone administered was sufficient to suppress endogenous corticoid production, so that the change effected by the estrogen must have involved an alteration of metabolism.

The effect of estrogen on protein binding of hydrocortisone

Normal values for ultrafiltrable hydrocortisone are shown in Figure 2, plotted against the corresponding normal values for total plasma hydrocortisone. The normal range for ultrafiltrable hydrocortisone is from 0.3 to 1.2 μg. per 100 ml. when the total ranges from 6 to 13 μg. per 100 ml. Most of the values for the proportion of ultrafiltrable material were between 5

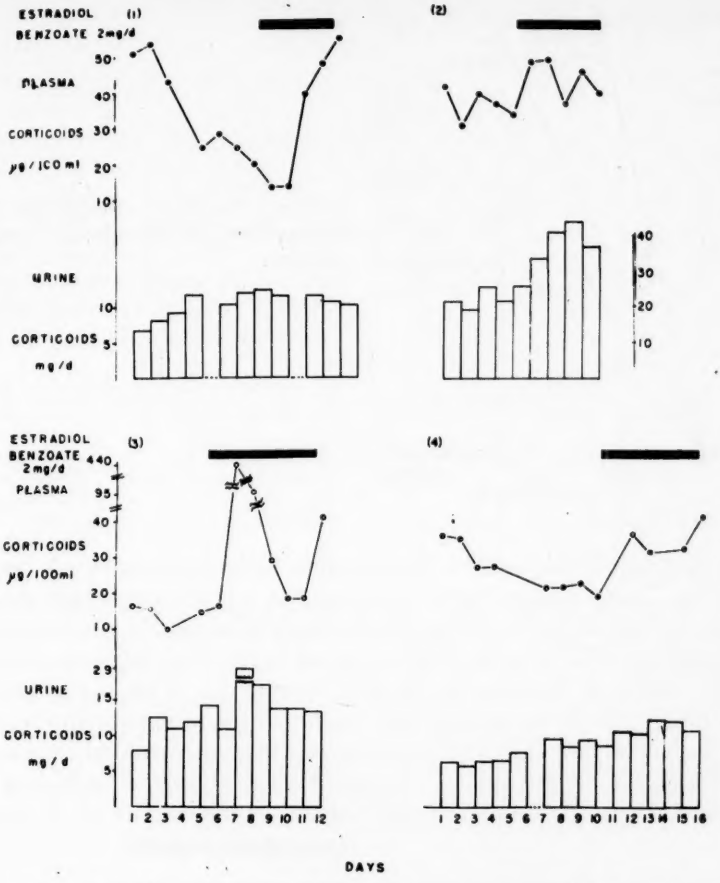

FIG. 1. The effect of intramuscular estradiol benzoate (2 mg. daily) on plasma and urinary 17-hydroxycorticosteroids during daily intramuscular injections of 100 mg. of cortisone acetate, in normal Subjects 1, 2 and 4 and in cirrhotic Subject 3.

and 10 per cent, with a few as low as 2.5 per cent and a few as high as 12.5 per cent.

The effects of estrogen on the level of total plasma hydrocortisone are shown in Table 1. The most striking results were in Subject W.S. The individual values for ultrafiltrable and total plasma hydrocortisone, and the 8 a.m. eosinophil counts, are presented in Figure 3. During the estrogen period there was a pronounced rise in the plasma level of total hydrocortisone, but in spite of this the eosinophils fell below the control values on only two days, and then not precipitously as one might expect on the basis

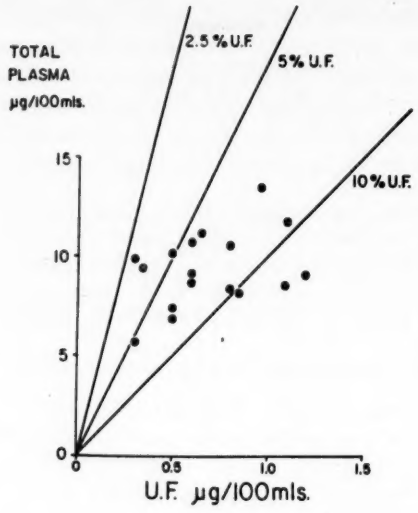

FIG. 2. Total and ultrafiltrable (U.F.) plasma hydrocortisone levels in normal subjects.

of these plasma values. The ultrafiltrable hydrocortisone levels, on the other hand, showed only slight variations, but it is of interest that they did rise above the control levels on the two days on which the eosinophils fell. No appreciable fall of eosinophils occurred in the other subjects. It would appear, therefore, that the rise in the concentration of plasma hydrocortisone brought about by estrogen was mainly in the protein-bound fraction. This is shown more clearly when the percentage of hydrocortisone that is ultrafiltrable is plotted against the total hydrocortisone, as in Figure 4 for Subject C.O. In Figure 5 are presented the combined data on 3 subjects,

TABLE 1. EFFECT OF ESTROGEN ADMINISTRATION* ON THE LEVEL OF TOTAL PLASMA HYDROCORTISONE

Subject	Plasma hydrocortisone level (µg./100 ml.)	
	Control	On 7th day of estrogen therapy
W.S., ♀	10.0	60.0
L.B., ♀	10.4	23.3
S.N., ♀	8.6	24.2
†R.L., ♂	5.0	13.7
C.O., ♀	11.1	36.0

* Dosage = 1.0 mg. of ethinyl estradiol daily, by mouth.
† Given 2 mg. of estradiol benzoate daily, by mouth.

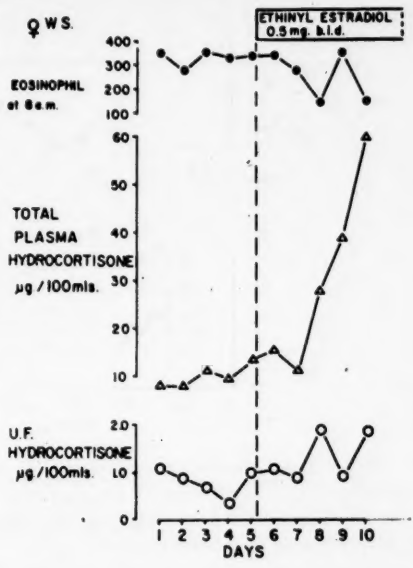

FIG. 3. Total and ultrafiltrable (U.F.) plasma hydrocortisone levels and 8 a.m. eosinophil counts before and during ethinyl estradiol therapy (1 mg./day) in Subject W.S., a normal 20-year-old woman.

prior to and during estrogen therapy. It is seen that as the total plasma level rises, the percentage of ultrafiltrable hydrocortisone falls. The mean percentage ultrafiltrable control value was 8.3, and the S.E. 0.87; during estrogen therapy the mean and S.E. were 5.4 and 0.43. The difference is 2.9, with an S.E. of 0.94. By the *t* test, this is highly significant, P being <0.01. This fall in the percentage of ultrafiltrable hydrocortisone is in contrast to what happens if hydrocortisone is added *in vitro* to control plasma and

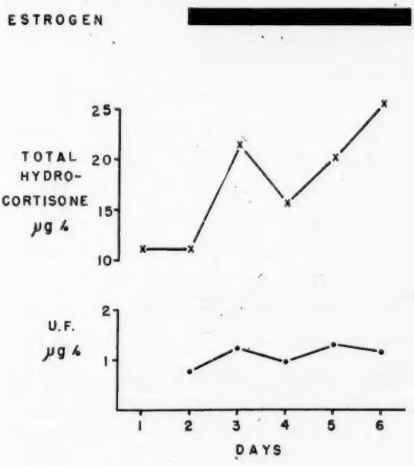

FIG. 4. Total and ultrafiltrable (U.F.) plasma hydrocortisone during administration of ethinyl estradiol (1 mg./day) to Subject C.O., a normal young woman.

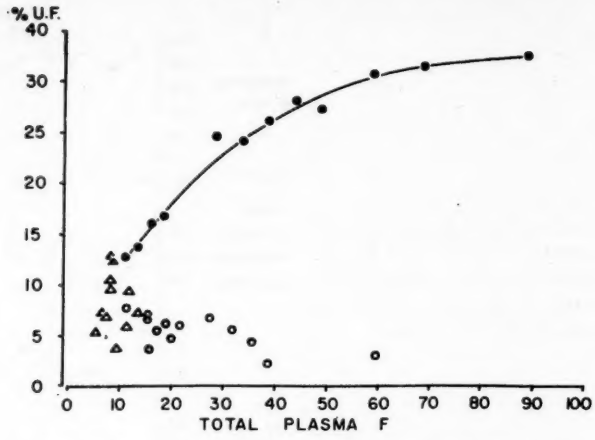

FIG. 5. Total plasma hydrocortisone (F) and percentage of ultrafiltrable (U.F.) hydrocortisone prior to (triangles) and during (circles) administration of ethinyl estradiol: combined data from 3 subjects. The black dots on the curve represent the effect of adding hydrocortisone to control plasma *in vitro*.

then ultrafiltered (Fig. 5, black dots on curve). In Figure 6 the fall in the percentage of hydrocortisone that is ultrafiltrable is shown for Subject S.N. using the *in vitro* technique and adding C^{14}-hydrocortisone (4 μg. per 100 ml.) to each sample. Some of the control values for the ultrafiltrable percentage were higher than usual, but at the outset the total plasma hydrocortisone level was 8.6 μg. and at the end it was 24.2 μg. per 100 ml.

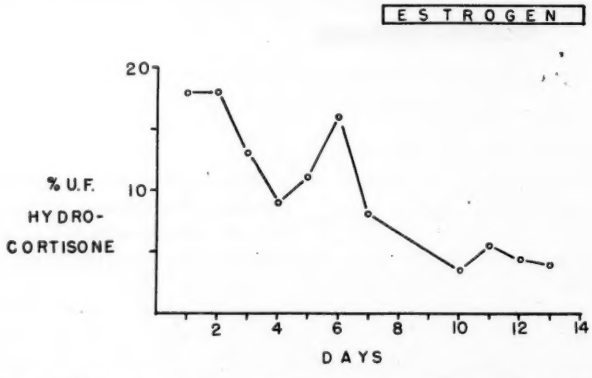

FIG. 6. The percentage of hydrocortisone that is ultrafiltrable (U.F.) determined by the *in vitro* technique prior to and during administration of ethinyl estradiol (1 mg./day) to Subject S.N., a normal young woman.

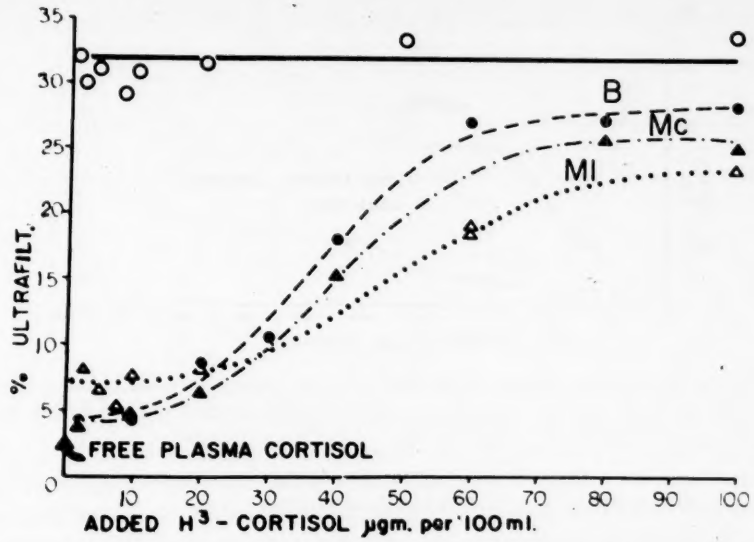

FIG. 7. The *in vitro* binding of hydrocortisone to plasma protein in 3 normal women whose plasma hydrocortisone had been suppressed with dexamethasone. The upper horizontal line (circles) is the curve obtained with purified human serum albumin.

With the concentration of total hydrocortisone rising and the percentage of ultrafiltrable hydrocortisone falling, the amount that was protein-bound must have risen.

The effect on the amount of hydrocortisone-binding protein or on the number of binding sites is clearly brought out by use of the *in vitro* technique with plasma from subjects in whom hydrocortisone production had been suppressed with dexamethasone. The results obtained in 3 normal young women are shown in Figure 7. Over the physiologic range of plasma hydrocortisone the binding was very high, but soon after this range was exceeded there was a rapid fall until a second plateau was reached which approached that derived from the binding by albumin alone. The first point of inflection in the curves reflects the amount of binding protein which was present. After treatment with estrogen there was a marked change in the curve, as shown in Figure 8. The capacity for binding hydrocortisone was now greatly increased, and it was not until more than 80 µg. of steroid per 100 ml. of plasma had been added that binding to albumin began. Even at 200 µg. per 100 ml. the hydrocortisone-binding protein was not completely saturated with steroid. These results cannot be explained by stronger binding at the same sites, because more steroid was now held by the high affinity protein.

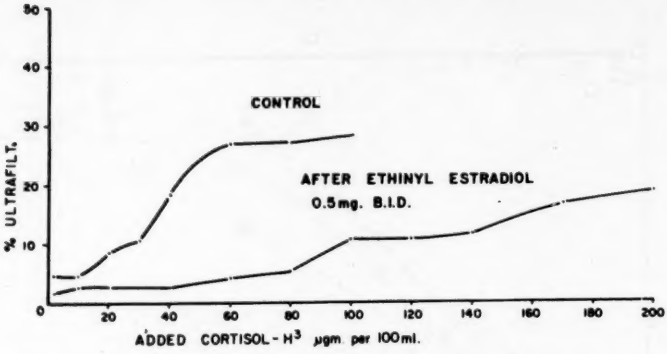

FIG. 8. The *in vitro* binding of hydrocortisone to plasma proteins before and after administration of ethinyl estradiol to a normal young woman (18 years old) whose plasma hydrocortisone has been suppressed with dexamethasone.

The rate of removal of hydrocortisone from plasma

In Figure 9 are shown the regression lines of radioactive hydrocortisone on time, plotted on semilog paper. The $t_d^{1/2}$ in the control period was 75 minutes and after administration of estrogen it increased to 114 minutes. The values for the $t_d^{1/2}$ in the control and estrogen periods are shown for 4 subjects in Table 2. The difference between the two sets of values is highly significant ($P < 0.01$). The administration of estrogen delayed the removal of hydrocortisone from the plasma.

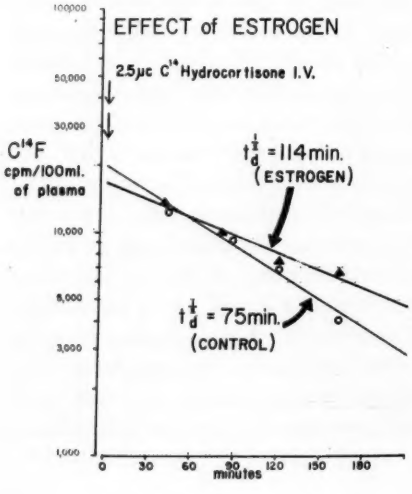

FIG. 9. The rate of removal of C^{14}-hydrocortisone from plasma before (circles) and after (triangles) administration of ethinyl estradiol to Subject C.O., a normal 20-year-old woman.

TABLE 2. HALF-TIME FOR DISAPPEARANCE ($t_d^{1/2}$) OF RADIOACTIVE HYDROCORTISONE FROM PLASMA, BEFORE AND AFTER ADMINISTRATION OF ESTROGEN

Subject	Half-time (mins.)		Difference (mins.)
	Control	After estrogen	
S.N.	60	126	66
L.B.	64	205	141
C.O.	75	114	39
W.S.	114	138	24

DISCUSSION

The administration of estrogen in comparatively large doses to both men and women produces variable increases in plasma hydrocortisone levels, regardless of whether the hydrocortisone is of endogenous or exogenous origin. Since the dose of cortisone given in the present studies was sufficient to suppress the production of corticotropin, it seems unlikely that the estrogen acted by stimulation of the pituitary, as suggested by Gemzell (7) and Taliaferro *et al.* (8). The demonstration that the level of plasma hydrocortisone of endogenous origin rises during estrogen therapy confirms reports by Taliaferro *et al.* (3) and Wallace *et al.* (4) that estrogens increase the level of Porter-Silber chromogens. Since the material was extracted and chromatographed in the present study, both as free steroid and as acetate, there is little doubt that the rise in corticosteroid concentration was due to hydrocortisone.

Results of the protein-binding studies are at variance with those reported by Daughaday (17) and by Sandberg *et al.* (18). However, their studies were carried out at 4° C., whereas ours were at 37° C. It was shown by Chen *et al.* (13) that the protein binding of hydrocortisone increases as the temperature is decreased, that ultrafiltration at 4° C. yields results comparable to those obtained by dialysis at this temperature (17, 18), and that dialysis at 37° C. yields binding values of the same order as those obtained by ultrafiltration. This temperature effect has recently been confirmed by Slaunwhite and Sandberg (19). Because of working at 4° C. with undiluted plasma, Daughaday was unable to demonstrate a rise in the hydrocortisone-binding protein in pregnancy (20), whereas this was demonstrated by Slaunwhite *et al.* (19). These observations and those of the present study support the view expressed by Martin and Mills (6) that the alteration of hydrocortisone metabolism during pregnancy might be the result of the high secretion of estrogen.

The changes in hydrocortisone metabolism brought about by estrogen include an increase in the hydrocortisone-binding protein or in the number of

binding sites, which raises the total hydrocortisone level without materially changing the nonprotein-bound fraction. The change in the rate of removal of hydrocortisone from the plasma during administration of estrogen suggests that the protein-bound steroid is protected to some extent from destruction by the liver.

The fact that the eosinophils are not depressed by the high levels of total hydrocortisone supports the view that only the nonprotein-bound steroid is biologically active. Peterson reported that patients with rheumatoid arthritis whose plasma hydrocortisone levels had been markedly raised by estrogen therapy experienced no remission of the disease (21). Since there is normally a diurnal variation in hydrocortisone secretion, with low production at night (22), the presence of a large amount of protein-bound hydrocortisone would act as a reserve when adrenal production falls. Conversely, a sharp rise in the secretion rate of hydrocortisone might have less biologic effect if there were a large amount of high-affinity protein capable of binding a correspondingly large portion of the steroid. It would appear, therefore, that the hydrocortisone-binding protein may act as a "buffer" to lessen the variations in the effective hydrocortisone level. In this respect it is similar to the thyroxine-binding protein. It is of interest that electrophoretically the thyroxine-binding protein runs in the same position as the hydrocortisone-binding protein (23, 17), and that the plasma protein-bound iodine is also raised by estrogen therapy (24). This may indicate that the same protein has binding sites for both hormones. It is unlikely that the same sites are involved, as thyroxine will not displace hydrocortisone from its binding protein (Chen, P. S. and Mills, I. H., unpublished observations).

The effect of estrogen in increasing the amount of hydrocortisone-binding protein raises the problem of the mechanism of control of the protein. Normally, the binding protein appears to be nearly saturated with hydrocortisone, but when the amount in circulation has been raised by administration of estrogen it has the ability to bind much more steroid than is present in the plasma. Consequently, during administration of corticotropin, exaggerated plasma responses occur. The data in Figure 1 show that urinary excretion of corticoids does not fall during the first few days of estrogen therapy. This is supported by the data of Peterson (21). However, in long-term estrogen studies there is a pronounced fall in urinary excretion of corticoid of either exogenous (2) or endogenous (21) origin. During this phase the plasma level changes very little (21). It is clear, therefore, that administration of estrogen has two distinct effects on hydrocortisone metabolism: 1) it causes a marked rise in the level of protein-bound steroid, and 2) it increases the total pool out of proportion to the rise in the level of plasma hydrocortisone (21) and simultaneously decreases the excretion of

metabolites. The effect on protein binding is independent of the pituitary (25) but the fall in urinary excretion is abolished (in guinea pigs) by hypophysectomy (26). The change in hydrocortisone metabolism which occurs in some persons during cortisone therapy (15) suggests that the binding protein may be depressed. Evidence for this has been found in 1 patient (Brooks, R. V. and Mills, I. H., unpublished observations). If this is a common occurrence in patients treated with cortisone, it is of considerable clinical importance. The relapse of various diseases when cortisone therapy is stopped might in part be related to the more rapid metabolism of endogenous hydrocortisone with effective (nonprotein-bound) plasma levels even lower than normal. This aspect of the problem is being further investigated.

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SUPPRESSION OF PITUITARY GONADOTROPINS BY 17-ETHYNYL-19-NORTESTOSTERONE IN PATIENTS WITH METASTATIC CARCINOMA OF THE BREAST

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ABSTRACT

17-Ethynyl-19-nortestosterone was found to be effective in suppressing pituitary gonadotropin excretion in 4 cases of metastatic mammary carcinoma. The mean 24-hour excretion of gonadotropin fell from 34.5 μg. to 7.7 μg. by the fourth week and to 5.7 μg. by the sixth week of injection, and remained low when the steroid was administered by mouth for an additional sixteen weeks. It is suggested that the effect might result from the weak estrogenic activity of the compound.

THERE is evidence that certain types of mammary carcinoma are stimulated by estrogens (1). A compound suppressing the gonadotropin output of the pituitary might obviate the use of oophorectomy and hypophysectomy in such cases.

A report is given of the urinary gonadotropin excretion in 5 cases of metastatic carcinoma of the breast treated with 17-ethynyl-19-nortestosterone over a period of six months.

MATERIALS AND METHODS

Patients and procedure

Two complete 24-hour specimens of urine were collected from each subject prior to treatment. After treatment had commenced, single 24-hour samples were collected at approximately weekly intervals for a period of six weeks. During this time each of the 5 patients received weekly injections of 100 mg. of 17-ethynyl-19-nortestosterone enanthate. Injections then ceased and the treatment was continued by oral administration of 15 mg. of 17-ethylnyl-19-nortestosterone acetate daily for a further sixteen weeks. Urine samples were collected from the subjects in only the fifteenth and sixteenth weeks of the oral treatment.

Four of the 5 patients had previously been oophorectomized; of these, 2 (K.R. and M.L.) had also been adrenalectomized, and 1 (E.B.) was adrenalectomized during the period of study. The remaining patient (M.R.) had received dienestrol therapy from 1953 to 1956, when it was suspended because of uterine bleeding and dermatitis.

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Extraction of gonadotropin

The gonadotropin was concentrated by the method of Loraine and Brown (2, 3), the crude extracts being stored until completion of the series. Immediately before the biologic assays were carried out, these extracts were further purified with tricalcium phosphate and reprecipitated with acetone (2, 3). For injection, the final precipitate was suspended in 4 ml. of water.

Biologic assay

(a) The urine extracts were assayed by the method of Claringbold and Lamond (4). Immature female mice (body weight 10–11 Gm.) received a single injection of 0.3 ml. of the extract. The animals were killed forty-four hours later; the uteri were removed, dried and weighed. The assay was carried out over four successive weeks, using one mouse per sample per week. For the purpose of analysis, the uterine weights were converted to logarithms.

(b) Estrogenic activity was measured by the method of Martin and Claringbold (5), which utilizes the mitogenic action of estrogens on the vaginal epithelium of the mouse. The compounds to be assayed were administered subcutaneously in oil to adult ovariectomized mice, as a single injection, and intravaginally in 25 per cent aqueous propyleneglycol. Colchicine (0.1 mg.) was injected after seventeen hours and the animals were killed at twenty-four hours. The vaginas were dissected out, fixed, sectioned, and the epithelial mitoses counted. For analysis the transformation 100 log (Z+2), where Z is the number of mitoses per field, was applied to the data.

RESULTS

Suppression of gonadotropin

Table 1 and Figure 1 show the gonadotropin excretion levels in 4 subjects before and after treatment. In the urine of 1 subject (M.R.) there was no detectable gonadotropin, perhaps due to previous estrogen therapy. An analysis of variance was carried out, from which Patient M.R. was omitted. Although this analysis showed a significant patient-by-treatment interaction, the linear treatment effect when tested against this interaction was highly significant ($P < 0.001$). The actual drop in excretion of gonadotropin was about eight- to ninefold. It is clear from the figure that administration of 17-ethynyl-19-nortestosterone enanthate, at the dosage level used, did not immediately reduce the secretion of gonadotropin. At least six weeks of treatment was necessary before the hormone levels approached a minimum. The low excretion of gonadotropin after sixteen weeks of oral administration of 17-ethynyl-19-nortestosterone acetate demonstrates that the steroid is active by this route.

Estrogenic activity of 17-ethynyl-19-nortestosterone

Table 2 shows the effect of 17-ethynyl-19-nortestosterone on mitotic activity in the vaginal epithelium of the ovariectomized mouse. It can be seen that the compound is weakly estrogenic, its potency in terms of estrone being 1.02×10^{-4} by the local route and 4×10^{-3} by the systemic

TABLE 1. URINARY GONADOTROPIN EXCRETION OF PATIENTS RECEIVING 17-ETHYNYL-19-NORTESTOSTERONE ENANTHATE AND ACETATE

Treatment commenced between weeks 1 and 2. The determinations are based on 4 animals each and negative values represent chance deviations from zero.

Weeks	Human pituitary gonadotropin excretion (mg./24 hrs.)				
	Patients				Mean
	K.R.	M.L.	J.W.	E.B.	
0	28.7	38.7	20.8	61.3	37.3
1	13.3	33.6	40.0	40.0	31.7
2	44.4	46.9	22.1	43.5	39.2
3	13.1	23.7	36.3	9.3	20.7
4	0.5	−3.5	20.8	26.7	11.3
5	−0.5	−0.5	6.9	25.1	7.7
6	2.4	+2.7	4.4	17.3	6.7
7	0.0	−4.0	9.3	17.3	5.7
22	−0.5	−4.0	26.7	−2.4	4.9
23	−3.2	−1.2	25.6	0.0	5.3

route. No significant differences were found between the dose-response slopes of the two compounds. The systemic local ratio of 43 for 17-ethynyl-19-nortestosterone compared with 1660 for estrone suggests that the activity of the former at least in part, is due to an estrogenic contaminant or to a

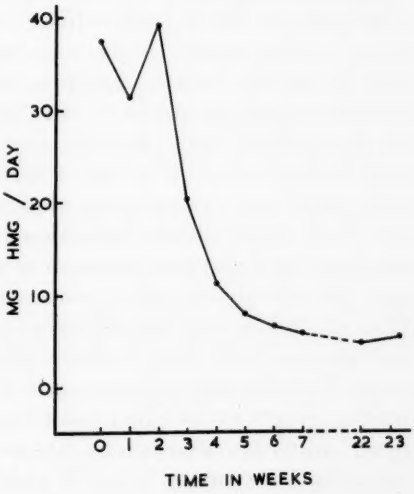

FIG. 1. Urinary gonadotropin (HMG) excretion in 4 cases of mammary carcinoma before and after treatment with 17-ethynyl-19-nortestosterone. Injections were commenced in the second week. The dotted portions of the graph represent the period of oral treatment, during which no samples were collected.

TABLE 2. EFFECT OF 17-ETHYNYL-19-NORTESTOSTERONE ACETATE ON THE MITOTIC ACTIVITY OF THE VAGINAL EPITHELIUM OF THE MOUSE

Route	Substance	Dose (μg.)	Mean No. of mitoses/field	No. of mice
Subcutaneous	Estrone	1×10^{-1}	22.5	6
		1×10^{-2}	6.2	4
	17-Ethynyl-19-nortestosetrone	1×10^{1}	18.7	6
		1×10^{0}	0.9	6
Intravaginal	Estrone	1×10^{-4}	28.7	5
		1×10^{-5}	7.9	6
	17-Ethynyl-19-nortestosterone	1×10^{1}	28.7	6
		1×10^{-1}	8.8	6

Systemic/local ratio	17-Ethynyl-19-nortestosterone	42.8(23.8−76.9; P=0.05)
	Estrone	1660(967−2910; P=0.05)
Relative potency of 17-ethynyl-19-nortestosterone in terms of estrone	Intravaginal, 1.02×10^{-4}	(0.66×10^{-4}−1.74×10^{-4}; P=0.05)
	Subcutaneous, 4×10^{-3}	(2.3×10^{-3}−6.9×10^{-3}; P=0.05)

direct action of the molecule, and possibly not to a metabolic transformation as in the case of a pro-estrogen (6).

DISCUSSION

Saunders and Drill (7) described 17-ethynyl-19-nortestosterone as a weak estrogen but did not estimate its potency. Pincus *et al.* (8), using the mouse uterine weight assay, reported its estrogenic activity as 1/10,000 that of estrone. Neither group noted any effect on the vagina, whereas Shipley [quoted by McGinty and Djerassi (9)], using the rat vaginal smear assay, found ethynyl-nortestosterone to have 1/250 the activity of estrone when administered by the subcutaneous route—a result which is in accord with the potency found in the present work. Assay of the estrogenic activity of 17-ethynyl-19-nortestosterone is complicated by the compound's strong progestational activity. It is well known that progesterone antagonizes the uterotropic action of estrogens (10), and that in the vagina conjoint administration with estrogens may result in mucification rather than cornification (10). Thus workers using slightly different criteria of scoring vaginal smears might obtain quite different estimates of potency, and the uterine weight assay might yield apparently low estimates of potency due to self-inhibition. The mitotic rate assay is not affected by simultaneous administration of progesterone in amounts 2,500 times the dose of estrogen

(Martin, in press), and might therefore be expected to provide a truer estimate of the estrogenic activity of 17-ethynyl-19-nortestosterone than other methods.

Goldman *et al.* (11) and McGinty and Djerassi (9) obtained suppression of pituitary gonadotropins in rats treated with 17-ethyl and 17-ethynyl-19-nortestosterone respectively, the former workers finding no evidence of estrogenic activity in their tests. These and the present data suggest that inhibition of pituitary gonadotropin secretion may be a general property of the 19-nortestosterones, although it is still not clear whether this results from their direct action or that of estrogenic metabolites or contaminants.

Acknowledgments

The authors wish to thank Professor C. W. Emmens for much helpful criticism and advice. The 17-ethynyl-19-nortestosterone was a gift from Schering, Berlin. The work was supported in part by a grant from the N.S.W. State Cancer Council.

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STEROID EXCRETION IN HYPOPHYSECTOMIZED WOMEN, AND THE INITIAL EFFECTS OF CORTICOTROPIN: A STUDY IN URINARY STEROID PATTERNS

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ABSTRACT

The relative dependence of adrenocortical biosynthetic pathways upon the action of ACTH has been examined by comprehensive analyses of urinary steroids in 4 hypophysectomized women. Urinary steroid patterns during adrenal insufficiency induced by the withdrawal of cortisone therapy were compared with those obtained before hypophysectomy and during the early phases of ACTH stimulation. The comprehensive analytical procedure of Wilson *et al.* was modified to separate metabolites of cortisol from those of corticosterone and 11-deoxycortisol. Measurable amounts of steroids were excreted during adrenocortical insufficiency, although in 2 patients subsequent sella reconstruction revealed that 95 to 98 per cent of the pituitary had been removed. Dehydroepiandrosterone and pregnanetriol, present in control urines, were not detectable after hypophysectomy. Paper chromatographic techniques showed the continued excretion of most other known major metabolites. Quantitative assay on column eluates indicated the 24-hour excretion of $C_{19}O_2$ fractions averaged 1.2 mg., $C_{19}O_3$ fractions 1.3 mg., and C_{21} cortisol metabolites 0.8 mg., or 30, 27 and 8 per cent respectively of the preoperative values. Thus secretion of cortisol was severely depressed, while C_{19} precursors continued at higher levels. The earliest and greatest effect of intravenous ACTH was to increase the output of cortisol metabolites. During the second twelve hours the increment was about three times that for the other fractions. The selective and profound effect of ACTH on cortisol production is consistent with current concepts that one action of this hormone is to promote adrenocortical hydroxylations, thereby favoring production of the most highly oxygenated corticoids. Formation of adrenal androgens appeared less dependent upon ACTH.

RESULTS of numerous animal experiments have indicated that the adrenal cortex secretes few, if any, measurable steroids following hypophysectomy (1–3). Similarly, analyses of crude extracts of urine from patients after hypophysectomy have shown very low titers (4, 5). Except

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for C_{19} steroids found by Holliday *et al.* (6), identifications of corticoid metabolites in the urine of completely hypophysectomized patients have not been reported.

The present study was initiated to ascertain whether the low titers of total steroids represent adrenocortical metabolites, to determine whether the pattern of excreted steroids is qualitatively altered, and to evaluate the early effects of adrenocorticotropin (ACTH) on a previously inactive adrenal cortex.

MATERIALS AND METHODS

Subjects. Four patients were studied. Control urines were collected on 3 patients before hypophysectomy. Approximately three weeks postoperatively, steroid therapy (cortisone) was withdrawn. After periods varying from five to nineteen days, signs of acute adrenocortical insufficiency developed, and plasma corticosteroid values fell almost to zero. The urine collected during the subsequent twelve to thirty hours was termed the "hypophysectomy" specimen. Intravenous ACTH was then given at the rate of 1 unit per hour for twenty-four hours, followed in 3 cases by intramuscular ACTH, 25 units every eight hours. Details of the urine collections are shown in Table 1.

Hydrolysis and extraction. The urine was incubated with β-glucuronidase ("Ketodase," Warner-Chilcott Co.) acidified to pH 0.8, and continuously extracted with ether. The first neutral fraction was prepared as previously described (7). The alkaline washes were brought to pH 0.8, and combined with the residual urine. The mixture was further acidified to pH 0, or an over-all concentration of 1 *N* sulfuric acid. It was again extracted for forty-eight hours. The first and second neutral fractions were combined.

Partition column chromatography. Portions of each extract representing no more than six-hour urine collections were chromatographed on a partition column using stepwise gradient elution (7). All solvents had been equilibrated with equal volumes of 50 per cent v/v aqueous ethanol. The following modified elution sequence was employed:

Eluate A. 100% hexane—($C_{19}O$, $C_{19}O_2$, and $C_{21}O_2$). Omitting the previous 2-ml. "front" collection, 3 fractions were collected in order to separate dehydroepiandrosterone (DHA).[1] These were:

A1, 0–15 ml. (including the pore volume).

[1] The following trivial names are used in the text:
Androsterone is 3α-hydroxyandrostane-17-one.
Etiocholanolone is 3α-hydroxyetiocholane-17-one.
Dehydroepiandrosterone (DHA) is Δ^5-3β-hydroxyandrostene-17-one.
11-Ketoetiocholanolone is 3α-hydroxyetiocholane-11,17-dione.
11β-Hydroxyetiocholanolone is 3α,11β-dihydroxyetiocholane-17-one.
11β-Hydroxyandrosterone is 3α,11β-dihydroxyandrostane-17-one.
Pregnanetriol is pregnane-3α,17α,20α-triol.
Δ^5-Pregnenetriol is Δ^5-pregnene-3β-17α,20α-triol.
11-Deoxycortisol (compound S) is 17α,21-dihydroxy-Δ^4-pregnene-3,20-dione.
Tetrahydro S is 3α,17α,21-trihydroxy-pregnane-20-one.
Cortisol (compound F) is 11β,17α,21-trihydroxy-Δ^4-pregnene-3,20-dione.
Tetrahydrocortisol is 3α,11β,17α-21-tetrahydroxypregnane-20-one.
Reichstein's substance E is 11β,17α,20β,21-tetrol-Δ^4-pregnene-3-one.
Cortols are pregnane-3α,11β,17α-20α(or β), 21-pentols.
Cortolones are 3α,17α,20α(or β)-tetrahydroxypregnane-11-ones.

TABLE 1. DATA ON 4 HYPOPHYSECTOMIZED WOMEN

	Subjects*			
	M.D., aged 59, post-menopausal	K.R., aged 49, ovariectomized	N.M., aged 45, ovariectomized	E.K., aged 51, post-menopausal
Days without cortisone**	11	5	19	11
Degree of adrenal insufficiency	Moderate	Moderate	Severe	Moderate
Total days after hypophysectomy†	33	50	43	33
Plasma corticosteroid level‡	4.3	0	1.6	0.7
Hours "hypophysectomy" urine	24	12	24	30
Hours of urine collections during 24-hour i.v. ACTH (1 unit per hour)	0–8 8–24	0–12 12–24	0–12 12–24	0–6 6–12 12–18 18–24
Hours of urine collections during i.m. ACTH (25 units per 8 hours)	80–96	84–96	24–36	—
Pituitary tissue at autopsy§	<2%		<5%	

* All subjects were women having cancer of the breast.

** Dosage of cortisone was 50 mg. per day, orally.

† The period during which the "hypophysectomy" urine was collected. A preoperative urine specimen was collected on all subjects except N.M.

‡ Silber-Porter chromogens (μg. per 100 ml. plasma).

§ We are indebted to Dr. John M. Van Buren of the National Institute of Neurological Diseases and Blindness for the information concerning the anatomic completeness of hypophysectomy.

A2, 15–40 ml. (all the DHA).
A3, 40–75 ml. (more polar $C_{19}O_2$ metabolites).
Eluate B. 5% $CHCl_3$:95% hexane—($C_{19}O_3$ and $C_{21}O_3$)—60 ml.
Eluate C. 15% $CHCl_3$:85% hexane—($C_{21}O_4$ and polar C_{19})—60 ml.
Eluate D. 30% $CHCl_3$:70% hexane—($C_{21}O_5$ and $C_{21}O_6$)—120 ml.
Eluate E. 50% $CHCl_3$:50% hexane—very polar corticosteroids—50 ml.

Tetrahydrocortisone is 3α,17α,21-trihydroxy-pregnane-11,20-dione.
21-Deoxycortisol is 11β-17α-dihydroxy-Δ^4-pregnene-3,20-dione.
Dihydrocortisone is 17α,21-dihydroxypregnane-3,11,20-trione.
Dihydrocortisol is 11β,17α,21-trihydroxypregnane-3,20-dione
Androstanedione is androstane-3,17-dione.
Etiocholanedione is etiocholane-3,17-dione.

The new fraction, eluate C, separates metabolites of precursors other than cortisol from the main cortisol metabolite fractions D and E. The known urinary steroids expected in this eluate occur in very small amounts, if at all, or are indicative of adrenocortical abnormality. They include compound S, tetrahydro S, and 3-keto-Δ^4-pregnen-17,20,21-triol, as well as corticosterone and its analogous metabolites, dihydrocortisone and dihydrocortisol, pregnane-3α,17α,20α-triol-11-one, and pregnane-3α,11β,17α,20α-tetrol. Some cortisone would be included, but no cortisol. Unknown polar C_{19} steroids are also present.

Quantitative colorimetric determinations. All urinary steroid values are expressed as mg. per twenty-four hours. All eluates were assayed by the CrO_3-KS reaction for total 17-ketosteroids (17-KS) and 17-hydroxycorticosteroids (7, 8). Values for eluates C, D and E in equivalents of oxidized androsterone were multiplied by 1.5 to convert them to mg. of C_{21} steroid and to correct for incomplete conversion to C_{19} 17-KS (8).

Eluates A3, B, C and D were assayed by the HIO_4-KS reaction for C_{21} 17,20-diols and 17,20,21-triols (9). Values in androsterone equivalents were calculated from the difference between oxidized and unoxidized samples, and were multiplied by 1.25 to give mg. of C_{21} steroids.[2] In eluate B, the $C_{19}O_3$ metabolites were estimated by subtracting the HIO_4-KS titer (representing pregnanetriol and other $C_{21}O_3$ 17,20-diols) from the CrO_3-KS value.

Eluates C, D and E were assayed by the Silber-Porter reaction (10).

Determination of dehydroepiandrosterone (DHA). Eluate A2, containing all the DHA, was treated with a solution containing 10 mg. of digitonin per ml. of 85 per cent ethanol, using 1 ml. of the solution per estimated mg. of steroid present. The precipitated 3β-hydroxy steroid fraction was recovered after decomposition with pyridine, and assayed for DHA by the Allen reaction (11).

Determination of pregnanediol. The portion of the A2 eluate not precipitated by digitonin (containing the pregnanediol) was acetylated, re-chromatographed, and the fractions containing pregnanediol diacetate assayed with concentrated sulfuric acid. Details of this procedure will be reported elsewhere.

Plasma corticosteroid levels were assayed by a modification of the Silber-Porter procedure (12).

Paper chromatography. Solvent systems: eluate A ($C_{19}O_2$) was run in iso-octane:80% methanol; eluate B in 50:50 iso-octane:toluene:50% methanol; eluate C in the Bush B_4 system (13); and eluates D and E in the Bush C system. Individual 17-KS were determined after eluting appropriate areas (14).

RESULTS

After hypophysectomy, when adrenal insufficiency became manifest following withdrawal of steroid therapy, most urinary titers fell to low levels, yet remained sufficient to demonstrate the continued secretion of adrenocorticosteroids.

When ACTH was administered intravenously, there was a refractory period of about eight hours before urinary steroid titers increased. During the second twelve hours, titers returned to approximately the control levels.

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[2] In this determination it was necessary to use a more concentrated HIO_4 reagent, 0.01 *M*, to obtain complete oxidation of pregnanetriol. The addition of $SnCl_2$ (9) should be omitted.

Hence, the adrenal cortices of these patients were capable of fairly prompt activation, even though they had been without pituitary corticotropin for five to seven weeks.

Excretion of various metabolites during the hypophysectomy period, as compared to control and ACTH periods

$C_{19}O_2$ steroids (eluate A). Average 24-hour total CrO_3-KS titers of eluates A1+A2+A3 fell from 4.0 mg. to 1.18 mg., *i.e.*, to about 30 per cent of control values; they were restored to 4.0 mg. by ACTH. Androsterone and etiocholanolone, determined individually after paper chromatography, were 20 per cent and 14 per cent of control values, respectively. Figure 1 compares the titers of these 2 metabolites with those of the total CrO_3-KS for the eluates in which they occurred, *viz*, A1 and A2α. That the uniformly

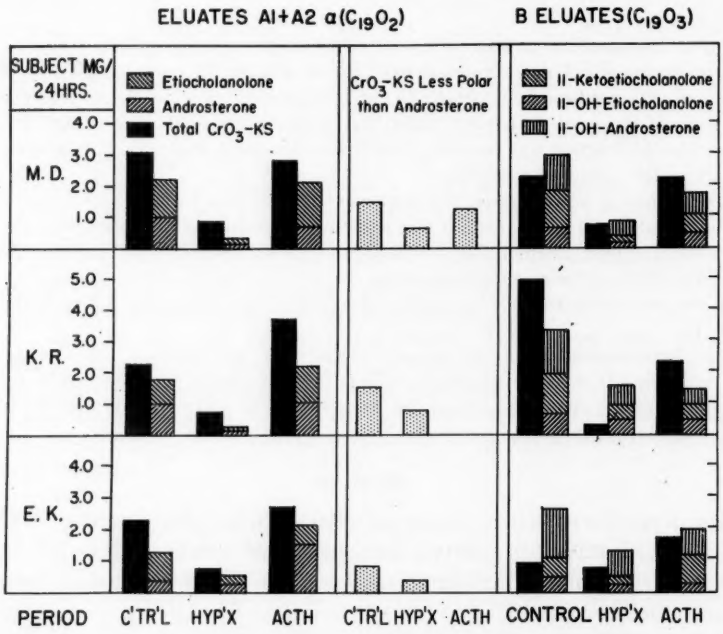

FIG. 1. Relationships between the CrO_3-KS titers of eluates from the partition column (height of each solid black bar) and the titers of individual components separated by paper chromatography (striped areas). The central section depicts the sum of assays on eluates of regions of the paper chromatograms other than those containing androsterone, etiocholanolone or DHA, which gave a positive reaction with alkaline m-dinitrobenzene on the paper after oxidation with CrO_3. Total CrO_3-KS titers of the B eluates were corrected for pregnanetriol.

higher value for CrO_3-KS reactants probably represents adrenocortical steroids and not extraneous chromogens is indicated by: 1) development of the typical bluish purple color; 2) recovery of the excess titer in Zimmermann-positive bands on papergrams run after chromic acid oxidation (Fig. 1, central section); 3) a decrease of this moiety during the hypophysectomy period; 4) the known presence of other $C_{19}O$ and $C_{19}O_2$ metabolites (15–18); and 5) the inclusion of nonketonic C_{19} metabolites in the CrO_3-KS determination.

Urinary DHA averaged less than 100 μg. per day preoperatively, fell to zero after hypophysectomy, and returned in small amounts when ACTH was given. These findings confirm those of Holliday *et al.* (6).

Pregnanediol excretion, determined quantitatively in 1 subject, fell from 20 μg. per day to zero, and was qualitatively very low in the other patients during the hypophysectomy period. Δ^5-Pregnenediol was never present in more than trace amounts, if at all.

$C_{19}O_3$ steroids (eluate B). Average total CrO_3-KS titers, corrected for 17,20-diols, fell from 2.8 mg. per day to 0.75 mg. (27 per cent of the control level). As is also shown in Figure 1, the 3 major C_{19} constituents of this fraction followed the same trend individually. With administration of ACTH, average titers returned to about 2.0 mg. per day.

Pregnanetriol and other $C_{21}O_3$ 17,20-diols (eluate B). Figure 2 shows that these diols were undetectable in the urine during the hypophysectomy period, whereas control levels had averaged 0.9 mg. per day. On giving ACTH these metabolites reappeared, usually in somewhat larger amounts. An unknown 17,20 diol was detected in eluate A3.

$C_{21}O_4$ and polar C_{19} steroids (eluate C). Figure 3 shows the changes in Silber-Porter (S-P) and HIO_4-KS reactants (largely $C_{21}O_4$ compounds of undetermined nature). A control average excretion of 0.64 mg. per day fell to 0.16 mg., *i.e.*, to 25 per cent. ACTH caused an elevation to 1.3 mg., or double the control level. It may therefore be concluded that after hypophysectomy there was at most a small extra production of either compound S, or of 21-deoxycortisol (the major metabolites of which are $C_{21}O_4$ 3,17,20-triols (19)).

The total CrO_3-KS titer of eluate C was not accounted for by the S-P and HIO_4-KS reactants (Fig. 3). This difference could be due to either C_{19} steroids or 21-deoxy-17-hydroxy-20-ketones. After reduction of these fractions with potassium borohydride, the HIO_4-KS titer remained far below that determined by CrO_3-KS. Hence, the difference must be due to unknown C_{19} steroids more polar than the usual $C_{19}O_3$ metabolites found in eluate B. This moiety averaged 1.64 mg. per day in control urines, fell to 0.50 mg. after hypophysectomy (30 per cent of the control), and returned to 1.66 mg. with ACTH.

EFFECT OF ACTH ON C_{21}-17,20-DIOLS

COLUMN ELUATE	SUBJECT	URINE COLLECTION				
		HYP'X	ACTH-1 I.V.	ACTH-2 I.V.	ACTH-3 I.M.	C'TR'L PRE-HYP
A3		UNKNOWN 17,20-diol				
	M.D.	0		0.11	0.07	0.26
	K.R.	0	0.19	0.05		0.33
	E.K.	0	Tr	0.38		0
	N.M.	0	0	0.38		
B		Pregnanetriol and Δ^5-Pregnenetriol				
	M.D.	0		0.81	0.94	1.7
	K.R.	0	0.20	1.1		Tr.
	E.K.	0	0.19	1.6		0.38
	N.M.	0	0	0.38		

FIG. 2. Excretion of C_{21}-17,20-diols during the hypophysectomy period, while the patients were receiving intravenous ACTH, and preoperatively. The values represent the differences between micro-Zimmermann assays with and without previous oxidation with periodic acid on eluate A3 (polar $C_{19}O_2$ and least polar $C_{21}O_3$) and eluate B ($C_{19}O_3$ and $C_{21}O_3$). The nature of the reacting material in eluate A3 is not known. In eluate B the titers represent chiefly pregnanetriol, plus any Δ^5-pregnenetriol which might be excreted. "Tr" indicates a trace of reactive material.

$C_{21}O_5$ and $C_{21}O_6$ steroids (eluates D and E). These 2 eluates contain all the known C_{21} metabolites of cortisol, unless dihydrocortisol or dihydrocortisone are excreted. Titers for the 2 fractions were therefore combined, and are shown in Figure 4. It is important to note the high proportion of HIO_4-KS reactants, unquestionably representing largely 17,20,21-triols. These accounted for 80 per cent of the total C_{21} cortisol metabolites in one instance. The sum of the HIO_4-KS and S-P assays is clearly a more accurate measure of C_{21} cortisol metabolites than either assay alone. The same value is usually obtained with the CrO_3-KS determination, which measures

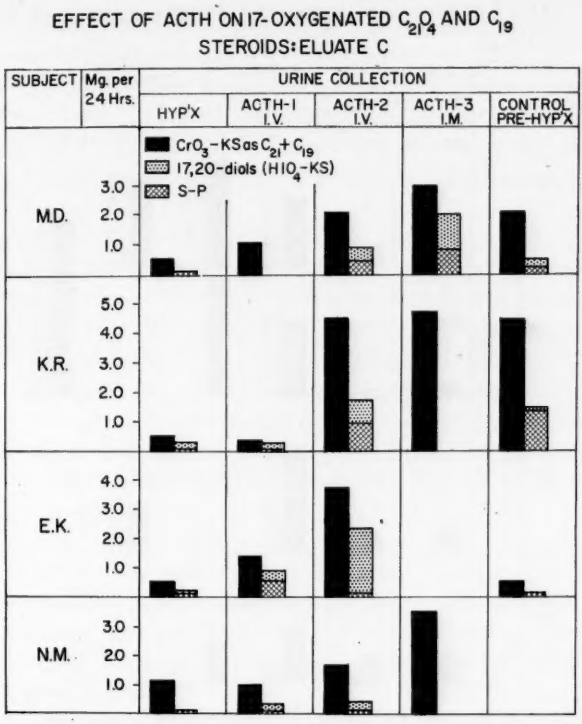

FIG. 3. Effect of hypophysectomy and of ACTH on the metabolites in eluate C. Total titers of 17-oxygenated "$C_{21}O_4$" steroids are equal to the sum of the 17,20-diols and Silber-Porter reactants. The amount by which the total CrO_3-KS value exceeds this sum represents chiefly C_{19} steroids. None of these titers can definitely be ascribed to known metabolites.

both classes of metabolites (Fig. 4). However, it can also be seen that other CrO_3-KS reactants may predominate at very low levels of cortisol secretion. This suggests the presence of either polar C_{19} steroids or of 21-deoxy-17-hydroxy-20-ketones which were not detected when ACTH was acting.

The total control excretion of metabolites of cortisol (HIO_4-KS+S-P; eluates D+E) averaged 13.3 mg. per day, and fell to 0.77 mg. (average fall = 92 per cent) after hypophysectomy. With ACTH this fraction increased about tenfold, to an average excretion of 7.2 mg. per day.

Change in ratio of C_{19} and cortisol metabolites. Before hypophysectomy the excretion of C_{21} metabolites of cortisol was two to five times as great as that of the $C_{19}O_2$ metabolites but, without ACTH, it fell to an average value below that for the $C_{19}O_2$ steroids. The average 92 per cent decrease in cor-

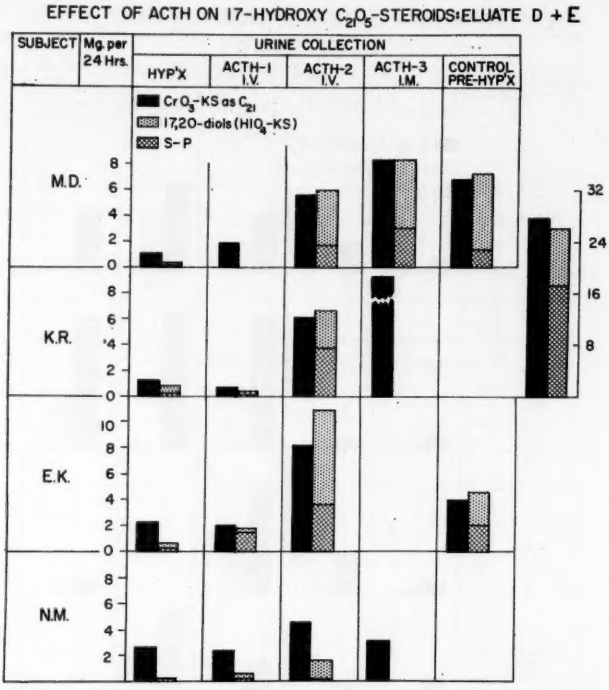

FIG. 4. Effect of hypophysectomy and of ACTH on the metabolites in eluates D+E. Total titers of 17-oxygenated "$C_{21}O_5$" and "$C_{21}O_6$" steroids are equal to the sum of the 17,20-diols (largely or entirely 17,20,21-triols) and Silber-Porter reactants. When ACTH was acting, the CrO_3-KS assay measured the same components (since it usually yielded the same value), but without ACTH the higher CrO_3-KS figure suggests the presence of other reactants.

tisol metabolites was 12 mg. per day, whereas each of the C_{19} fractions declined by roughly 3 mg., or 70 per cent. When ACTH was administered, the metabolites of cortisol again predominated.

Paper chromatographic characterization of metabolites of cortisol during the hypophysectomy period. The validity of the colorimetric determinations on eluates D and E was confirmed by finding areas on paper chromatograms which reacted to appropriate tests for α-ketols opposite tetrahydrocortisone and tetrahydrocortisol reference standards. Oxidation with periodic acid also yielded areas opposite a cortolone standard giving the Zimmermann reaction.

DISCUSSION

Our analyses have demonstrated that in hypophysectomized women a small but definite secretion of adrenocortical steroids continues. The com-

pleteness of hypophysectomy (proved at autopsy in 2 of the 4 women) as well as the marked signs of adrenal insufficiency, are evidence that ACTH was virtually absent. The possibility that metabolites of administered steroids were being measured seems unlikely. Hellman *et al.* (20) have shown that orally administered C^{14}-cortisol is completely or largely excreted in four days; in our subjects the withdrawal periods ranged from five to nineteen days.

Several previous investigators have observed that ACTH given to normal persons produces a greater increase in urinary corticosteroids than in "total 17-ketosteroids" (21–23). Our studies define this phenomenon further by showing that after removal of most or all of the pituitary ACTH secretion the $C_{19}O_2$ and the $C_{19}O_3$ urinary steroids decrease to about a third of control values, whereas C_{21} metabolites of cortisol decrease to less than a tenth of control values. Conversely, restoration of ACTH to the point of a normal level of steroidogenesis quickly produces a sharp selective increase in the urinary metabolites of cortisol, several-fold greater than that of other adrenocortical steroids.

This effect is consistent with the concept that one action of ACTH is the promotion of all the biosynthetic hydroxylation reactions by indirectly increasing the availability of a common co-factor, *viz*, reduced triphosphopyridine nucleotide (24, 25). Thus, ACTH drives the biosynthetic mechanism to completion, resulting in a large production of the most highly oxygenated corticoid, cortisol.

It seems most likely that ACTH also promotes the formation of Δ^5-pregnenolone from cholesterol (26). If this reaction could proceed without ACTH, one would expect a secretion of Δ^5-pregnenolone and progesterone. However, little or no Δ^5-pregnenediol or pregnanediol, the major metabolites of these precursors, was excreted in the urine; hence they did not accumulate in the absence of ACTH. Since 2 other intermediates, 17-hydroxyprogesterone and compound S, did not accumulate either, all processes in the pathway of cortisol formation appear to have been reduced to negligible levels.

At the same time, production of $C_{19}O_2$ steroids proceeded to a greater extent without ACTH, and was increased to a lesser extent by restoration of ACTH than was the formation of cortisol. One $C_{19}O_2$ precursor, Δ^4-androstenedione, has been shown to arise from 17-hydroxyprogesterone in the adrenal cortex (27, 28). One would expect this reaction to be sharply curtailed in the absence of ACTH (29). Our data therefore support the existence of an alternative route, possibly from DHA (30). Whichever be correct, the reaction sequences leading to adrenal $C_{19}O_2$ steroids appear less dependent upon ACTH than do those leading to cortisol.

No gross block in the adrenocortical biosynthetic sequence was detected when ACTH was given after several weeks of adrenal inactivity. Metabo-

lites of abnormal products such as 17-hydroxyprogesterone or compound S were only slightly if at all increased. On the contrary, the normal end-product, cortisol, was promptly formed.

These studies also emphasize that chromatographic analyses specific for urinary metabolites of cortisol provide a sensitive index of its rate of secretion when processes such as renal function are normal.

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TRIIODOTHYRONINE IN THE DIAGNOSIS AND TREATMENT OF HYPOTHYROIDISM: FAILURE TO DEMONSTRATE THE METABOLIC INSUFFICIENCY SYNDROME (CONTROLLED STUDY)

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ABSTRACT

This study fails to confirm the entity of metabolic insufficiency, in which patients are said to show symptoms suggesting hypothyroidism, a low basal metabolic rate, normal thyroid function by other tests, and a response to triiodothyronine but not to desiccated thyroid. In a double-blind study of 20 cases, as many patients improved during treatment with placebo as with triiodothyronine. In 12 patients with normal levels of serum protein-bound iodine, the control of fatigue and elevation of BMR were as satisfactory with desiccated thyroid as with triiodothyronine when equivalent doses were used. Triiodothyronine was used in therapeutic trials in 132 cases of suspected hypothyroidism. It has advantages for such use because of the rapidity of onset and cessation of its action. Thus an acceptable BMR level is reached within two weeks and symptoms of overdosage are readily recognized. The dose varied from 25 to 150 μg. An overdose resulted more often in nervousness than in cardiac consciousness. Desiccated thyroid was preferred for long-term use.

THE clinical usefulness of triiodothyronine has gradually been defined since its identification, isolation and synthesis in 1952–1953 (1, 2). Comparable metabolic effects are achieved in myxedema by triiodothyronine, thyroxine or desiccated thyroid (3–7) but triiodothyronine has a more rapid onset and cessation of action (4, 8–11). A dose of 30 micrograms of *l*-triiodothyronine is equivalent to 1 grain of desiccated thyroid (9). Additional current knowledge and theory regarding triiodothyronine have been reviewed elsewhere (12–15). It has been postulated that thyroxine is deiodinated in the tissues to triiodothyronine and that the latter is the intracellular form of the thyroid hormone.

The concept of metabolic insufficiency or nonmyxedematous hypometabolism has recently been revived. Such patients are said to show symptoms suggestive of hypothyroidism, a low basal metabolic rate, normal thyroid function by other tests, failure to respond to desiccated thyroid, but clini-

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cal improvement when treated with triiodothyronine. About 45 such cases have been reported (14, 16, 17). Additional studies are difficult to evaluate (18–21). This concept of metabolic insufficiency has been challenged (15, 22, 23). Controlled studies have been suggested, but no reports of such studies have been found.

Although there is no agreement about the existence of an entity labeled metabolic insufficiency and the allegedly indispensable role of triiodothyronine in its treatment, triiodothyronine is reported (15) to be a valuable substitute for desiccated thyroid or thyroxine, chiefly because of its speed of action. A therapeutic test in equivocal hypothyroidism can be accomplished more quickly and clearly. Speed may also be an advantage in the diagnosis of hyperthyroidism[1] and in treatment of myxedematous coma, myxedematous mania or thyroid carcinoma between doses of radioiodine.

It was the purpose of this study to test some of these concepts.

The subjects were students seen by one physician in the medical clinic of the University of Minnesota Health Service. Basal metabolic rates (BMR) were determined in duplicate by either of two technicians. Serum protein-bound iodine (PBI) determinations were carried out in duplicate at the University of Minnesota Hospitals, using a modification of the method of Barker *et al.* (24). The study consisted of three parts.

Group A: Controlled (double-blind) study of 20 cases of fatigue and low basal metabolic rate treated with triiodothyronine

Materials and methods

This group was composed of 20 students (1 man and 19 women) aged 17 to 30 years. The chief complaint of fatigue did not seem to be adequately explained by the history, the results of physical examination and other laboratory studies, nor by the schedule and personal habits. Some subjects had constipation, muscular aches, intolerance of cold, or irritability. Basal metabolic rates were moderately low. The student was informed that the study was for the purpose of comparing two thyroid products. Before treatment, the basal metabolic rate and the serum protein-bound iodine level were determined. Data were recorded initially and at intervals on a special study sheet regarding the amount of fatigue, hours of sleep, daytime drowsiness, work load (academic credits, extra-curricular activities, outside job), emotional factors, additional medications, amount of coffee consumed, and miscellaneous factors. A summary was made at each visit.

Triiodothyronine[2] and placebo tablets were dispensed by the usual double-blind method, except that physician and patient knew when the medication was changed

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[1] Werner test.

[2] 3:5:3′-*l*-Triiodothyronine was supplied as Cytomel, and placebo tablets of the 25-μg. size were supplied as Cytomel Placebo through the courtesy of Smith, Kline, and French Laboratories, Philadelphia, Pa.

because of the need to select the individually required dose. Each student took the entire daily dose in the morning, beginning with 50 μg. The daily dose was increased by 25 to 50 μg. each week until optimum control of fatigue was secured, or to a maximum of 150 μg. If symptoms of overdosage occurred, the medication was discontinued for two days then resumed at the highest well tolerated dose.

Each student continued to use the selected dose and reported for basal metabolic rate and clinical evaluation at two, four and six weeks. The serum protein-bound iodine level was determined at six weeks. The procedure was then repeated with the second medication.

Results (Group A)

Table 1 shows the clinical results in the double-blind study on triiodothyronine and on placebo, *each compared with no treatment.* There was no significant difference. In the *comparison of the two medications,* 9 students were better when taking triiodothyronine, 10 were better when taking placebo and 1 felt equally well with each. Table 2 presents laboratory data with cases separated as to clinical results, and shows that there was little difference between the two groups. With triiodothyronine, the average BMR for all cases was −4 per cent at two weeks, −5 per cent at four weeks and −6 per cent at six weeks.

Nineteen subjects had normal serum PBI levels initially and when they were taking placebo. One patient had an initial PBI level of 2.8 μg. per 100 ml., which rose to 5.4 μg. with placebo. There was poor agreement in 8 cases between the initial and the placebo PBI levels—a difference of 1.1 to

TABLE 1. CLINICAL RESULTS OF TREATMENT IN 20 CASES OF FATIGUE

	Improved	Partially improved	Equivocal	Unimproved
Triiodothyronine	10	7	1	2
Placebo	9	6	2	3

TABLE 2. LABORATORY DATA IN 20 CASES OF FATIGUE

Results of treatment	No. of cases	Before treatment		Triiodothyronine		Placebo	
			BMR (%)	PBI (μg./100ml.)*	BMR (%)	PBI (μg./100ml.)	BMR (%)†	PBI (μg./100 ml.)
Better with triiodothryonine than with placebo	9	range	−14 to −18	3.8 to 6.6	+3 to −16	1.3 to 3.5	−7 to −18	4.0 to 5.5
		avge.	−16	5.2	−4	2.3	−12	4.7
Better with placebo than with triiodothyronine	10	range	−12 to −23	2.8‡ to 7.4	+6 to −18	2.2 to 3.9	+6 to −27	3.8 to 6.3
		avge.	−16	5.2	−7	2.7	−12	5.0

* Normal range for this laboratory is 3.5 to 8.0 μg. per 100 ml. of serum.
† Average of BMR's at two, four and six weeks of treatment in each case.
‡ One case, 2.8 μg.; others, 3.8 to 7.4 μg. per 100 ml.

2.6 μg. per 100 ml. This resulted in a different classification (normal *vs.* low) in only the 1 case. There was a regular and significant lowering of the PBI level during triiodothyronine therapy.

Dosages of triiodothyronine varied widely. The effective daily dose in improved cases was 62.5 to 150 μg. This was also the highest well tolerated dose in unimproved cases.

Discussion (Group A)

The 20 subjects in the controlled study had the characteristics ascribed to alleged metabolic insufficiency. They had fatigue and other symptoms suggestive of hypothyroidism. Each had a moderately low BMR and a normal level of serum PBI.

Response to treatment showed that as many patients were better when taking placebo as when taking triiodothyronine. It would seem that either these patients did not have metabolic insufficiency, or that a beneficial effect of triiodothyronine on metabolic insufficiency cannot be shown by a double-blind study.

Group B: Clinical experience in a larger group of patients treated with triiodothyronine (no controls)

Materials and methods

Following the controlled study, additional students who would previously have been treated with desiccated thyroid were treated instead with triiodothyronine.

There were 132 such students who underwent adequate trial of therapy. The group was predominantly female and aged 18 to 22 years for the most part.

Their symptoms were sufficiently suggestive of hypothyroidism combined with a low BMR to make a trial of treatment worth while. Specific symptoms were fatigue or menstrual disorders, or both. Fatigue included undue exhaustion, lethargy, sluggishness, lack of alertness, drowsiness or an occasional feeling of depression. Menstrual disorders included infrequent, irregular or excessive flows in patients with normal pelvic findings.

The patient was told that his symptoms could plausibly be expected to respond to triiodothyronine but that a therapeutic trial with an open mind was indicated. The usual starting dosage was 50 μg. daily, but it was 25 μg. when the BMR was −25 per cent or lower, and 12.5 μg. in 1 case of myxedema. Usually the daily dose was increased by 25 μg. each week to an optimal level for control of fatigue or to a maximum of 150 μg. In later cases the maximum was 125 μg. If symptoms of overdosage occurred, treatment was omitted for two days and resumed at a level of 25 μg. less per day. In cases of menstrual disorders without accompanying fatigue, the desired daily dose was defined as 25 μg. less than that causing toxic symptoms. The selected dose was used for two weeks, the BMR determination was repeated and the dose was readjusted if indicated. In 38 of the cases of hypothyroidism with fatigue, therapy was later changed to equivalent doses of desiccated thyroid.

Results (Group B)

Clinical results related to the presenting symptoms are shown in Table 3.

TABLE 3. CLINICAL RESULTS RELATED TO PRESENTING SYMPTOMS IN PATIENTS TREATED WITH TRIIODOTHRYONINE

Clinical result	Fatigue	Fatigue & menstrual disorder	Menstrual disorder	Total
Equivocal response	15	0	6	21
Unimproved	27	0	11	38
Improved	47	8	18	73
Totals	89	8	35	132

Of the 132 students, 73 (55 per cent) exhibited convincing improvement when treated with triiodothyronine, and their cases were diagnosed as hypothyroidism. One of them had myxedema. The 38 unimproved students were regarded as euthyroid. Approximately the same percentage of improvement was noted in the fatigue group as in the menstrual group.

Initial basal metabolic rates varied from −33 to −12 per cent, most of them being in the range from −22 to −15 per cent. The distribution was similar in the hypothyroid and euthyroid groups. Initial serum protein-bound iodine levels were determined in 28 cases; they were normal in 21 instances.

The dosage of triiodothyronine (Fig. 1) required in the hypothyroid cases varied from 25 to 150 μg. daily. The most common daily dose was 100 μg. The highest well tolerated daily dose in the euthyroid cases was 25 to 150 μg. The required or tolerated dose was a highly individual matter. There was no correlation between the initial BMR and the effective dose of triiodothyronine (Fig. 2). Most patients receiving triiodothyronine had a BMR between −1 and −14 per cent. The range was similar in the hypothyroid and euthyroid groups.

Symptoms of overdosage occurred in 37 per cent of the hypothyroid and in 42 per cent of the euthyroid subjects. The specific symptoms in order of decreasing frequency were:

Nervousness, restlessness	38 cases
Tachycardia and/or palpitation	16
Insomnia	8
Headache	6
Increased fatigue	6

These were noted chiefly two to four days after starting an increased dosage schedule, and they disappeared in one or two days when treatment was temporarily discontinued. They were easily recognized by the student and were a good guide to dosage. The optimal daily dose was 25 μg. (rarely 12.5 μg.) less than that leading to symptoms of overdosage.

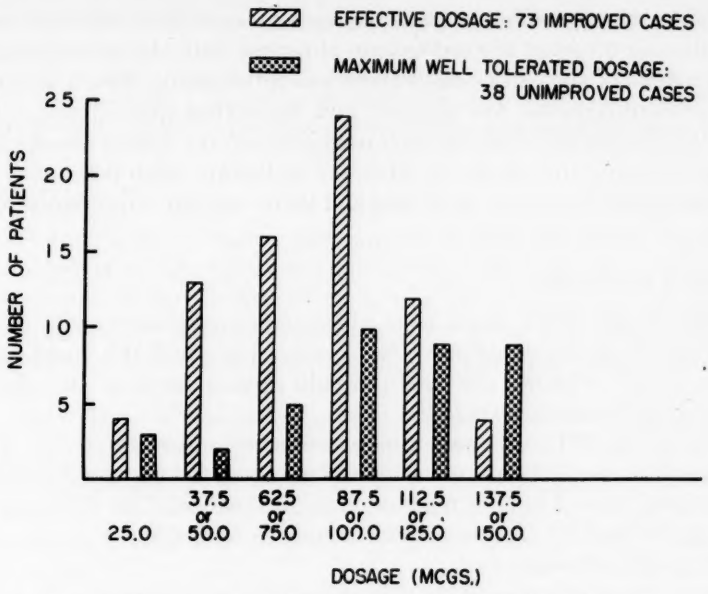

FIG. 1. Relationship of triiodothyronine dosage to clinical response.

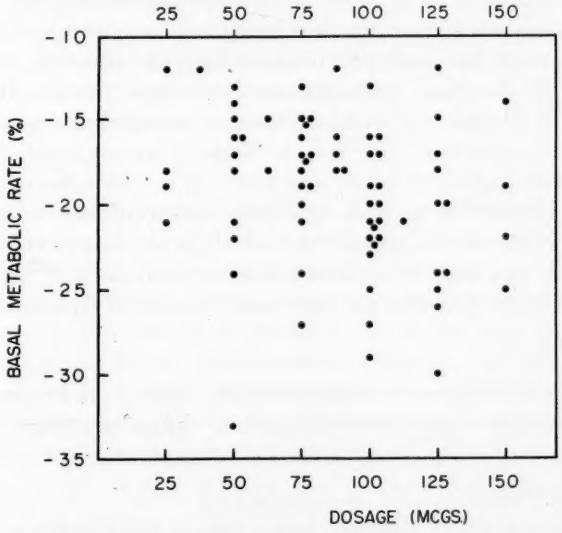

FIG. 2. Lack of correlation between initial basal metabolic rate and effective dosage of triiodothyronine (73 cases).

Subjects whose medication was changed to equivalent doses of desiccated thyroid reported as good control of fatigue with the second preparation. In some of the earlier cases there was a temporary return of fatigue when triiodothyronine was stopped and desiccated thyroid was substituted. An overlap schedule was later used in which the dose of triiodothyronine was tapered to zero by the fifth day of therapy with desiccated thyroid; this prevented return of fatigue and there were no symptoms of overdosage.

Discussion (Group B)

The low initial BMR in subjects with symptoms served chiefly to indicate the need for a therapeutic trial. The level of the BMR could not be used to predict whether the patient would respond or what the effective dose of triiodothyronine would be.

Initial serum PBI levels were usually normal when tested (Groups A and B). Therefore the PBI determination did not seem to be a useful screening test in these cases of mild to moderate hypothyroidism. The PBI level was regularly lowered by treatment with triiodothyronine, but this was of no help in judging dosage.

The results of therapeutic trials in these cases were more clear-cut than results of similar trials with desiccated thyroid in previous groups of students. The advantage of obtaining a follow-up BMR determination at two weeks rather than at two months, as with desiccated thyroid, is obvious. However, desiccated thyroid was preferred for long-term use because of more stable control, familiarity to all physicians, and economy. It should be emphasized that the daily starting dose, the increments and the effective dose found suitable for this group of healthy young students with mild to moderate hypothyroidism are not necessarily recommended for other groups of different ages and types, and certainly not for cases of myxedema.

Symptoms of overdosage with triiodothyronine do not seem to be entirely the same as with desiccated thyroid. With the former the overdosage was frequently manifested as nervousness without cardiac consciousness, whereas with desiccated thyroid the most common symptom is related to the heart.

Group C: Clinical experience in a group of patients responding to triiodothyronine and later treated with desiccated thyroid

Materials and methods

Seventeen subjects with a chief complaint of fatigue, who had improved during triiodothyronine therapy, were transferred to desiccated thyroid therapy in order to obtain adequate data for comparison of the two medications. After control of fatigue and ele-

vation of the BMR, triiodothyronine was discontinued for two weeks to observe whether the patient had a clinical relapse, and to secure serum PBI and BMR determinations. The equivalent dosage of desiccated thyroid was then started at once without stepwise trials in order to obtain a comparison of dosage without an undue time interval. After two months of desiccated thyroid therapy, the BMR and clinical observations were repeated.

Results (Group C)

Table 4 presents laboratory data on the 17 students treated with equivalent doses of triiodothyronine and desiccated thyroid. The BMR levels before treatment were similar to those two weeks after discontinuing triiodothyronine. The degree of elevation of the BMR was similar with the two products. The serum PBI concentration was normal in 12 of the cases.

TABLE 4. DATA ON 17 SUBJECTS TREATED WITH TRIIODOTHYRONINE AND LATER WITH DESICCATED THYROID

	Before treatment	Triiodothyronine		No treatment		Desiccated thyroid	
	BMR (%)	Daily dose (μg.)	BMR (%)	PBI (μg./100 ml.)	BMR (%)	Daily dose (gr.)	BMR (%)
Range	−13 to −27	50 to 150	+6 to −18	2.4 to 6.4	−13 to −24	1½ to 4	+7 to −19
Average	−19	95	−6	4.5	−18	3	−6

Clinically each patient experienced a relapse during the two weeks without treatment. In 1 case the relapse consisted of fatigue and moderately severe depression—the symptoms which had previously responded to triiodothyronine. Each patient reported comparable control of fatigue when equivalent doses of desiccated thyroid were substituted.

Discussion (Group C)

The 12 subjects with normal serum PBI levels are of special interest. Because of this finding, they would be classified by some workers as cases of metabolic insufficiency. Yet in all of them there was a good response to desiccated thyroid, contrary to the concept of metabolic insufficiency. This was a small group, but the results indicate that failure to respond to desiccated thyroid is not an invariable result when the serum PBI level is normal. It seems best to regard these cases as representing mild to moderate hypothyroidism, and to consider the BMR superior to the PBI determination as a screening test in such instances.

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A COMPARISON OF THE EARLY METABOLIC EFFECTS OF TRIIODOTHYRONINE AND THYROTROPIC HORMONE IN MAN*

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ABSTRACT

The early metabolic effects of sodium *l*-triiodothyronine (T_3) and thyrotropic hormone (TSH) were compared within an eight-hour period. Observations were made on 5 healthy volunteer male medical students on each of three occasions after fasting overnight. Each student received TSH (0.44 unit of Thytropar per Kg.) intramuscularly; T_3 (0.5 or 1.0 mg.) intravenously as a single dose; and, as a control, intravenous or intramuscular saline in an amount similar to that administered with the TSH or T_3. The subjects lay recumbent, dozing and reading at intervals over the eight-hour period. A significantly greater rise in the basal metabolic rate was noted following TSH than following T_3 (0.5 or 1.0 mg.). This rise was accompanied by increases in pulse rate and pulse pressure—which were not observed following T_3. T_3, however, produced a significant increase in the levels of plasma and urinary inorganic phosphate—which was not observed following TSH. The early metabolic effect of TSH was significantly less in a group of 5 hypothyroid subjects maintained with desiccated thyroid. It is concluded that the early metabolic effect of TSH differs from that of T_3, but apparently is of thyroid origin. This may possibly be due to the secretion of a rapidly acting hormone distinct from triiodothyronine.

AT PRESENT the physiologic significance of triiodothyronine remains uncertain (1). The original evidence of Gross and Leblond in animals (2) and Pitt-Rivers *et al.* in man (3) that triiodothyronine was formed in the tissues by the deiodination of thyroxine has not been confirmed by a recent study (4). Roche and Michel (5) have failed to demonstrate the formation of triiodothyronine in the peripheral tissues, and have concluded that the thyroid gland is the only source of circulating triiodothyronine. The findings of Taurog *et al.* in sheep (6) suggest that the thyroid gland secretes triiodothyronine. However, no increase in this hormone has been found following administration of thyroid-stimulating hormone (TSH) to euthy-

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roid subjects although such an increase was observed in hyperthyroid patients (7).

In previous reports (8–10) rapid metabolic changes, possibly of thyroid origin, have been described in association with emotionally stressful life experiences in man. At that time these findings raised the question of the possible mechanism of the participation of the thyroid in such rapid changes—in particular, whether triiodothyronine was secreted under these conditions, and how rapidly metabolic changes occurred following administration of triiodothyronine and thyrotropic hormone.

It was decided, therefore, to make a study of the early metabolic effects of these two agents. Each was given to the same subjects so that a comparison of their effects could be made. In order to determine whether TSH produced any metabolic effect of extrathyroidal origin, studies were also carried out in hypothyroid subjects maintained with desiccated thyroid. A preliminary report on these data has already appeared (11).

METHODS

Observations were made on 5 healthy volunteer male medical students on each of three occasions after fasting overnight. Each student received thyrotropic hormone[1] (TSH) intramuscularly (mean dosage 0.44 U.S.P. unit per Kg. dissolved in 4 ml. of saline (see Table 4); sodium *l*-triiodothyronine[2] (T_3), 0.5 or 1.0 mg. intravenously as a single dose; and, as a control, saline (physiologic sodium chloride) intravenously or intramuscularly in amounts similar to those administered with TSH or T_3. The subjects lay recumbent, dozing and reading at intervals over the eight-hour period.

The observations on the metabolic rate (MR), respiratory quotient, pulse rate and blood pressure were made every two hours for eight hours. The metabolic rate was determined using the Hartmann-Braun apparatus. With this open-circuit instrument, the values for O_2 consumption and CO_2 elimination are read directly from a hot wire and Wheatstone Bridge circuit. On each occasion observations were made at one-minute intervals for ten minutes and the mean values taken. The metabolic rate was then calculated according to a standard formula. Results obtained with this machine were found to be in satisfactory agreement with those obtained with the Jones machine. The nonprotein respiratory quotient (NPRQ) was also calculated.

Samples of blood and urine were collected on arrival of the student at the laboratory between 8 a.m. and 9 a.m. and then at two-hour intervals for eight hours. The concentration of plasma protein-bound iodine (PBI) was determined by the alkaline ash method as modified by Acland (12). Total nitrogen excretion was determined by the micro-Kjeldahl method with Nesslerisation, sodium and potassium with the EEL flame photometer, inorganic phosphate by the method of Taussky and Shorr (13), and creatinine by the method of Bonsnes and Taussky (14).

Similar observations were made on a series of 5 hypothyroid subjects maintained with desiccated thyroid (BP). The following are the clinical details on these patients.

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[1] "Thytropar," kindly supplied by Armour Pharmaceutical Company, Kankakee, Illinois. Samples with Batch numbers N2211, M2004, P2308, R2402, R2506 were used.

[2] Kindly supplied by Glaxo Laboratories, Greenford, Middlesex, England.

B.P., a woman aged 34, had undergone hemithyroidectomy for papillary adenocarcinoma of the thyroid four years previously, followed by a course of radiation postoperatively. She had eventually become hypothyroid (BMR−22 per cent, serum cholesterol level 405 mg. per 100 ml.) and had been taking thyroid (2 grains daily) for two months.

L.T., a man aged 55, had undergone hemithyroidectomy for carcinoma of the thyroid fourteen years previously, followed by deep x-ray treatment. Seven years later symptoms of hypothyroidism developed, and he had been receiving thyroid for three years—in a dosage of 3 grains daily for two years.

D.B. was a woman aged 39. Hypothyroidism had developed following the birth of her third child (BMR−35 per cent, serum cholesterol level 355 mg. per 100 ml.). Gonadal and adrenal functions were normal. She had been receiving 3 grains of thyroid daily for two months.,

R.M., a man aged 55, presented a classic case of myxedema (BMR−33 per cent, serum cholesterol level 398 mg. per 100 ml.). He had been receiving 2½ grains of thyroid daily for two months.

M.R. was a woman aged 38. Myxedema had developed following subtotal thyroidectomy for toxic goiter twenty years before (serum cholesterol level 335 mg. per 100 ml., BMR−45 per cent). She had been receiving thyroid (3 grains daily) for six months.

RESULTS

A. *Comparison of early metabolic effects of T_3 and TSH in 5 euthyroid subjects.*

Clinical effects. Two of the subjects complained of headache two hours after the administration of TSH, 1 had nausea, and 2 were asymptomatic. No symptoms were reported following triiodothyronine.

Effects on general metabolic rate and pattern. As shown in Table 1 and Figure 1, the MR rose in 4 of the 5 subjects, five to seven hours following administration of TSH. Administration of T_3 (0.5 or 1.0 mg.) produced an elevation in 3 of the 5 subjects in the same period ($P=0.07$). Statistical analysis indicated a significantly greater rise following TSH ($P<0.01$). Calculation of the NPRQ revealed a fall in 4 of the 5 subjects within three hours following administration of TSH. This fall, however, was not maintained. It was not observed after T_3 in this investigation although it has been observed previously (15). It may be seen that even under control conditions there was a tendency for the metabolic rate to increase during the latter half of the eight-hour period of observation. This was attributed to some degree of restlessness in the subjects, associated with forced recumbency. It has been previously noted under these conditions (15).

Effects on pulse rate and pulse pressure. Increases in pulse rate were consistently observed following TSH, but not following T_3 ($P<0.01$) (Table 2, Fig. 1). Observations on blood pressure in 3 of these subjects revealed a coincident rise in pulse pressure following TSH, but not following T_3. There was, however, no correlation between the increase in metabolic rate and the increase in pulse rate following TSH in these instances.

TABLE 1. EFFECTS OF TSH AND T_3 ON THE METABOLIC RATE IN 5 NORMAL SUBJECTS
(calories per square meter per hour)

Subject	Treatment	Time in hours			
		1	3	5	7
K.Y.	TSH	36.7	38.7	43.1	44.2
	T_3 (1.0 mg.)	37.3	39.3	40.0	42.2
	Saline	37.5	39.2	38.1	41.3
J.K.	TSH	38.7	41.7	45.4	49.9
	T_3 (1.0 mg.)	36.6	39.7	40.5	40.7
	Saline	37.6	37.8	39.8	40.6
L.M.	TSH	37.5	41.3	44.6	46.1
	T_3 (0.5 mg.)	38.5	38.1	40.0	42.6
	Saline	41.0	40.4	41.9	43.0
Q.M.	TSH	36.6	38.3	41.3	41.7
	T_3 (0.5 mg.)	43.0	44.3	46.6	48.1
	Saline	41.1	41.6	44.5	43.0
B.R.	TSH	38.9	42.6	45.0	47.5
	T_3 (0.5 mg.)	37.5	—	43.9	43.3
	Saline	36.7	37.1	38.8	40.0
Mean values	TSH	38.9	41.1	44.0	46.6
	T_3	40.2	42.0	43.3	44.5
	Saline	40.2	40.5	41.7	42.3

Statistical analysis using trend analysis showed a significantly greater rise following TSH when compared with T_3 and saline ($P < 0.01$), and following T_3 when compared with saline ($P < 0.07$).

Effects on plasma and urinary inorganic phosphate. A striking increase in both plasma and urinary inorganic phosphate resulted from the administration of T_3. No rise occurred following TSH (Table 3, Fig. 1).

Effects on other urinary constituents. Transient increases in sodium and potassium excretion were noted within four hours after administration of TSH ($P < 0.05$). No such changes occurred following T_3. No consistent changes in urine flow, or in total nitrogen or creatinine excretion occurred with either agent.

B. *Comparison of effects of TSH in 5 euthyroid and 5 hypothyroid subjects*

Clinical effects. Three of the hypothyroid patients complained of headache during treatment with saline as well as with TSH; the other 2 were

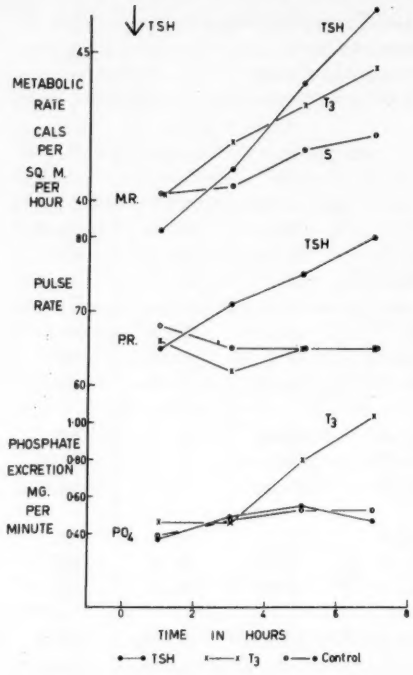

FIG. 1. Comparison of the effects of triiodothyronine (T_3), thyrotropic hormone (TSH), and saline (S) in 5 normal subjects. (M.R. = metabolic rate; P.R. = pulse rate; PO_4 = phosphate excretion.)

asymptomatic. In general, the subjective disturbance appeared less than in the euthyroid subjects.

Effect on plasma PBI level. Following administration of TSH in euthyroid subjects there was a striking increase in the PBI level (mean 3.2 μg. per 100 ml.) within eight hours; this increase had become evident by four hours (Table 4). On the other hand, in the hypothyroid subjects maintained with desiccated thyroid there was no rise in plasma PBI concentration with the same dose of TSH (Table 4, Fig. 2).

TABLE 2. MEAN PULSE RATE IN 5 NORMAL SUBJECTS GIVEN TSH AND T_3

Treatment	Time in hours				P*
	1	3	5	7	
TSH	65	72	75	80	0.01
T_3	66	62	65	65	N.S.†
Saline	68	65	65	65	—

* Probability, using trend analysis of difference from control.
† Not significant.

TABLE 3

(a) EFFECT OF TSH AND T_3 ON PLASMA INORGANIC PHOSPHATE LEVEL (MG./100 ML.)
(mean values for 5 subjects)

Treatment	Time in hours		
	0	4	8
TSH	4.5	4.6	4.6
T_3	4.7	5.1	5.8***
Saline	5.0	4.8	4.7

(b) EFFECT OF TSH AND T_3 ON URINARY INORGANIC PHOSPHATE (MG. PER MIN.)
(mean values for 5 subjects)

Treatment	Time in hours			
	0–2	2–4	4–6	6–8
TSH	0.37	0.49	0.55	0.47
T_3	0.46	0.46	0.80*	1.04**
Saline	0.39	0.47	0.53	0.53

* $P < 0.05$
** $P < 0.025$ } compared with saline, using "*t*" test.
*** $P < 0.001$

Effect on metabolic rate. The effect of TSH on the MR in the 5 hypothyroid subjects maintained with thyroid is shown in Table 5. An increase was noted in 2 subjects (L.T. and D.B.), but there was no overall increase and the changes were significantly less than those observed in the euthyroid subjects given the same dosage ($P < 0.001$). The mean increase in metabolic rate was proportionately greater in the normal subjects (21.6 per cent) than in the hypothyroid patients (8.3 per cent). There was a tendency for the metabolic rate to increase in the latter half of the eight-hour period under control conditions, as was noted in the normal subjects (Fig. 2).

Effects on pulse rate and pulse pressure. The effect of TSH on the mean pulse rate in 4 of the 5 hypothyroid patients maintained with thyroid is shown in Table 6. An increase was noted in 2 subjects—those showing an increase in the metabolic rate (L.T. and D.B.). One of these (D.B.) also showed an increase in pulse pressure. This change was less than in the euthyroid subjects given the same dose ($P < 0.07$).

Effects on urinary constituents. There was a retention of water and sodium

TABLE 4. EFFECT OF TSH ON PLASMA PBI LEVEL

Subject	Treatment	Dose of TSH*	Time in hours		
			0	4	8
Euthyroid Subjects					
K.Y.	TSH	0.52	6.4	7.6	8.6
	Saline		5.4	5.7	5.4
J.K.	TSH	0.44	3.8	6.1	6.4
	Saline		3.8	4.1	3.4
L.M.	TSH	0.43	4.3	4.9	7.3
	Saline		4.4	4.5	3.9
Q.M.	TSH	0.38	5.1	6.3	8.3
	Saline		—	5.1	5.0
B.R.	TSH	0.45	4.8	5.7	8.7
	Saline		4.8	4.3	5.8
Mean values	TSH	0.44	4.9	6.1	7.9
	Saline		4.6	4.7	4.7
Hypothyroid Subjects Maintained with Thyroid					
B.P.	TSH	0.44	5.2	4.0	4.4
	Saline		5.0	—	6.1
L.T.	TSH	0.50	2.7	—	2.8
	Saline		2.6	3.0	2.8
D.B.	TSH	0.39	2.9	2.6	3.1
	Saline		2.9	2.7	3.5
R.M.	TSH	0.43	2.1	—	2.0
	Saline		2.1	—	2.0
M.R.	TSH	0.42	2.8	2.7	2.8
	Saline		3.7	3.7	3.5
Mean values	TSH	0.44	3.1	—	3.0
	Saline		3.3	—	3.6

* Dose per kilo in U.S.P. units.

TABLE 5. EFFECT OF TSH ON THE METABOLIC RATE IN 5 HYPOTHYROID SUBJECTS MAINTAINED WTIH THYROID

(calories per square meter per hour)

Subject	Treatment	Time in hours			
		1	3	5	7
B.P.	TSH	33.8	38.3	37.8	36.3
	Saline (S)	37.9	35.9	36.9	38.6
L.T.	TSH	39.0	39.3	40.9	42.7
	Saline	34.9	39.1	39.0	39.3
D.B.	TSH	32.5	30.6	35.4	37.1
	Saline	33.0	31.9	33.3	33.6
R.M.	TSH	30.0	29.1	30.7	31.6
	Saline	29.2	24.9	28.7	30.3
M.R.	TSH	35.1	33.3	35.1	36.6
	Saline	36.9	35.9	37.6	39.2
Mean values	TSH	34.0	34.2	36.0	36.9
	Saline	34.4	33.5	35.1	36.2

Statistical analysis showed no significant difference between the effect of TSH and that of the control (S).

Comparison with the effect of TSH in normal subjects, using trend analysis, revealed a highly significant difference ($P < 0.001$).

TABLE 6. MEAN PULSE RATE IN 4 HYPOTHYROID SUBJECTS WHEN GIVEN TSH AND WHEN GIVEN SALINE ONLY (CONTROL)

Treatment	Time in hours				P*
	1	3	5	7	
TSH	61	64	66	69	N.S.†
Saline	63	62	62	64	—

* Probability, using trend analysis of difference from control.

Comparison of rise in euthyroid subjects (Table 2) with that in hypothyroid subjects ($P < 0.07$).

† Not significant.

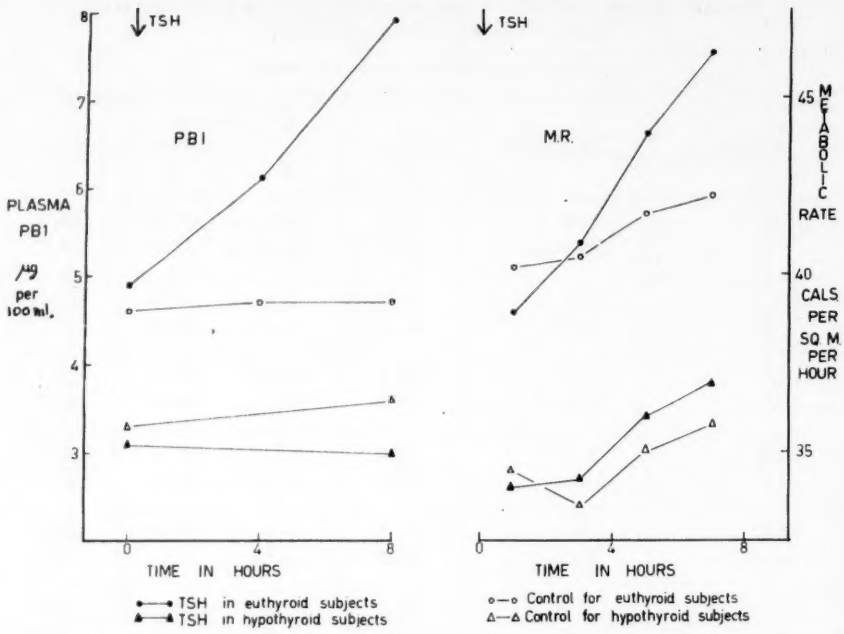

FIG. 2. Comparison of the effects of thyrotropic hormone (TSH) on the plasma PBI level and metabolic rate (M.R.) in euthyroid and hypothyroid subjects (mean values for 5 subjects in each group).

during the first four hours in all 5 hypothyroid patients following administration of TSH. This was followed by increased loss of potassium within four to six hours in 4 of the 5 patients. However, these changes were not statistically significant. They were attributed to a nonspecific "stress" effect.

DISCUSSION

The changes following administration of T_3 are those which have been previously reported from this laboratory, and are similar to those noted by others, occurring over a longer period of observation (15–17). In the first eight hours the striking feature is the increase in the excretion of inorganic phosphorus. There is no apparent difference between the effects of 0.5 mg. and 1.0 mg. of triiodothyronine given intravenously over this period of time (15). The increase in inorganic phosphorus excretion has been shown previously to be accompanied by an increase in the concentration of serum inorganic phosphorus (15) and this was confirmed in the present study. The increase is evident without an increase in total nitrogen excretion, indicating the independence of this effect from the breakdown of nitrogen. It would appear more likely to be related to the breakdown of high-energy

phosphate, perhaps associated with uncoupling of oxidative phosphorylation (17, 18).

Comparison of the early effects of T_3 with those of TSH reveals some striking differences (Fig. 1). Unlike the results following T_3, an increase in pulse rate and pulse pressure was observed within eight hours following TSH[3]. No correlation between this increase in pulse rate and the rise in metabolic rate was found in the individual subjects. The transient increases in sodium and potassium excretion noted within the first four hours after TSH were possibly associated with this increase in pulse rate and pulse pressure (19). However, the phosphate diuresis observed following T_3 did not occur following TSH in spite of a greater increase in metabolic rate following TSH. Neither was any rise in the level of plasma phosphate observed following TSH. This finding suggests a qualitative difference between the effects of the two agents.

However, the nature of these changes following TSH is such as to raise the question of their relation to stimulation of the thyroid. The increase in metabolic rate, pulse rate and pulse pressure could be part of a nonspecific reaction to the parenteral administration of protein. The transient changes in sodium and potassium excretion might result from the hemodynamic changes, and the absence of a phosphate diuresis—a characteristic effect of T_3, T_4, triac and tetrac (16, 17, 20)—is evidence against thyroid activation.

The question would be answered by a study of the effects of this TSH preparation in athyrotic subjects. Such patients were not available, but an attempt to meet this requirement was made by studies in hypothyroid patients receiving a maintenance dose of desiccated thyroid. These subjects had previous definite evidence of subnormal thyroid function and observations were made after correction of their hypothyroid state with replacement therapy had begun. This would minimize nonspecific factors operating to produce differences between such patients and normal subjects. In fact the differences between the mean initial metabolic rates were found to be of the order of 15 per cent in the two groups.

There was no rise in the plasma PBI level within eight hours after administration of TSH in the hypothyroid group, in marked contrast to the result in the euthyroid subjects (Table 4), indicating that the hypothyroid patients approximated the athyrotic state over this period. There was no increase in the metabolic rate or pulse rate in 3 of these subjects; however, increases in the metabolic rate were noted in the 2 others (L.T. and D.B.). The consistent changes in the urinary constituents in the hypothyroid subjects suggest a nonspecific "stress" effect which was not apparent in the normal group. Statistical analysis of the overall trend in the hypothyroid

[3] Note added in proof: A recent study in this laboratory (Wellby *et al.*, to be published) has demonstrated the same pattern of changes following administration of a different preparation of TSH ("Primothyron," Schering, A. G., Berlin) in equivalent dosage.

group revealed no significant increase in the metabolic rate and a significant decrease when compared with the euthyroid group.

It might be suggested that such a difference could be due to an inability of the hypothyroid patients to exhibit a nonspecific metabolic response. However, salicylate causes a marked increase in the metabolic rate in myxedematous patients, similar to that observed in euthyroid subjects (21). Moreover, hypothyroid patients are more sensitive to thyroid hormones than are normal subjects (22). It seems likely, therefore, that there would be no difference in the metabolic effect of TSH in the euthyroid and hypothyroid groups, if it were of extrathyroidal origin. The positive response in 2 of the hypothyroid patients may indicate a nonspecific component in this increase or some degree of thyroid stimulation, even though there was no significant elevation of the plasma PBI level during the period of observation. Emphasis is laid on the highly significant difference in the response of the two groups, rather than on the variable effects in individual hypothyroid (but not athyrotic) patients.

In view of all these considerations it is suggested that this early effect of TSH is of thyroid origin and, therefore, may be due to a rapidly acting agent distinct from triiodothyronine (11, 23). This agent would presumably be more potent than T_3, because it would appear unlikely that amounts of the order of 0.5 mg. would be secreted within a few hours as a result of stimulation with TSH. In this connection it is of interest that a recent chromatographic study failed to reveal an increase in T_3 output in euthyroid subjects given TSH, though such a change could be shown in hyperthyroid subjects (7). Another recent chromatographic study provides evidence of a circulating hormone distinct from T_3 and T_4 (24).

There is other metabolic evidence of a rapidly acting thyroid hormone distinct from triiodothyronine, based on previous observations made during stressful life experiences in man (8–10). These studies revealed a rapid increase in the MR and a fall in the NPRQ with an increase in total nitrogen, sodium and potassium (but not phosphate) excretion which was less marked in hypothyroid subjects maintained with desiccated thyroid, *i.e.*, a pattern of changes similar to those described here following administration of TSH.

These differences between the early effects of TSH and those of T_3 recall the previously described greater calorigenic effect of desiccated thyroid than that indicated by its "thyroxine iodine content" (25). Similar findings have been reported with thyroglobulin, which do not appear to be due to differences in the rate of absorption (26, 27). Such discrepancies also appear to be too great to be accounted for by the minute amount of triiodothyronine recovered from the thyroid (28).

In a subsequent study (29) a comparison has been made between the

early metabolic effects of large single doses of thyroxine (2.5–3.0 mg.) and desiccated thyroid (1.3–2.0 Gm.) sufficient to cause elevation of the plasma PBI level to 9–10 μg. per 100 ml. within two hours and the effects of T_3 in 0.5-mg. dosage. Neither of these agents reproduced the early metabolic effect of TSH.

All these reports, together with the observations recorded here, indicate that the precise nature of the physiologically active form of the thyroid hormone is still uncertain. This applies both to the thyroid secretion, which may vary under different conditions, and to the form in which the hormone is active in the peripheral tissues. In this connection, the significance of possible alterations in plasma protein binding of the thyroid hormones (30) has yet to be explored.

Acknowledgments

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SERUM CONCENTRATION AND THYROIDAL UPTAKE RATE OF STABLE IODIDE IN VARIOUS STATES OF THYROID FUNCTION*

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ABSTRACT

The thyroidal uptake rate of stable iodide is equal to the product of the thyroidal clearance rate of iodide and the serum concentration of stable iodide. A new method is reported for the determination of serum iodide concentration from the specific activity of saliva iodine. The determined values of serum iodide concentration are smaller than those reported by others, but there was no significant difference in a series of 16 patients with various states of thyroid function. Daily hormone formation and release may be calculated from the thyroidal uptake rate of stable iodide. By comparison of the quantitative results of iodide metabolism with the clinical state, evaluation of effective thyroid function may be possible under various clinical or experimental conditions. The thyroidal radioiodide accumulation rate is a function of the thyroidal clearance rate of iodide, *i.e.*, the capacity of the total gland to accumulate iodide. However, neither of these rates reflects the actual uptake rate of stable iodide in every case, owing to varying serum iodide concentration.

THE ability of thyroid gland to accumulate iodide may be investigated by labeling plasma iodide with radioiodide as an indicator. However, radioiodide accumulation tests furnish no information regarding the amount of stable iodide taken up by the gland per unit of time. The purpose of this paper is to report a new method for determination of the concentration of inorganic iodide in serum and of the rate of thyroidal uptake of stable iodide in human subjects, using radioiodide^{131}. Preliminary results of these investigations have been reported (1, 2).

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METHODS AND SUBJECTS

Subjects having normal thyroid function or untreated toxic goiter and those with such miscellaneous disorders of the thyroid as previously treated hyperthyroidism, iodine-deficient goiter, nontoxic nodular goiter or carcinoma of the thyroid, were evaluated clinically by the usual criteria of history and physical examination. The state of thyroid function was determined by measuring the basal metabolic rate (BMR) and the concentration of protein-bound iodine in serum (PBI).

Carrier-free iodide^{131} was then injected into the fasting subjects intravenously, the dose ranging from 20 to 40 microcuries (μc.). During the following two hours thyroidal radioiodide accumulation was measured every ten minutes, using a highly sensitive 5-cm. lead-shielded gamma scintillation counter at a skin-crystal distance of 45 cm., with a lead collimator from crystal to neck; scattered radiation by Compton-effects was eliminated. The radioiodide accumulation rate, corrected for background and physical decay, was determined as the percentage of the dose per minute and plotted against the time after injection.

At 30, 60, 90 and 120 minutes after the injection, samples of blood and samples of mixed saliva (stimulated by crystals of iodine-free citric acid and continuously secreted and withdrawn some minutes before and during the real collection period of three minutes) were assayed for radioiodide concentration, using a highly sensitive well-type scintillation counter, and corrected for physical decay. In order to calculate the specific activity of saliva iodine, the concentration of stable iodine in the saliva samples was determined microchemically (3); recovery of iodine was checked by measuring the radioactivity of the samples before and after digestion and distillation (3). The radioactivity of serum iodide (per cent of dose per ml.) and the specific activity of mixed-saliva iodine (per cent of dose per μg.) were plotted against the time after injection.

From these data and curves, the serum concentration and the thyroidal uptake rate of stable iodide were calculated. In the following equations:

T = a 30-minute period from 60 to 90 minutes following injection.

Thy.^{131} = mean thyroidal radioiodide accumulation rate in T (per cent of dose per minute).

Thy.^{127} = mean thyroidal uptake rate of stable iodide in T (μg. per minute).

Ser.^{131} = mean serum concentration of radioiodide in T (per cent of dose per ml), calculated from the time-serum iodide^{131} curve.

Ser.^{127} = mean serum concentration of stable iodide in T (μg. per ml).

Sal.^{131} = mean saliva concentration of radioiodide in T (per cent of dose per ml).

Sal.^{127} = mean saliva concentration of stable iodide in T (μg. per ml).

$\frac{\text{Sal.}^{131}}{\text{Sal.}^{127}}$ = mean specific activity of saliva iodide in T (per cent of dose per μg.), calculated from the time-specific activity curve of saliva iodide.

After injection, there is a progressively decreasing specific activity of serum iodide. The thyroid gland takes up serum iodide. Consequently, the specific activity of accumulated iodide is equal to the specific activity of serum iodide at any given moment. Therefore, at the mid-point of the short time interval T:

$$\text{I.} \qquad \frac{\text{Thy.}^{131}\ (\%\ \text{of dose/min.})}{\text{Thy.}^{127}\ (\mu\text{g./min.})} = \frac{\text{Ser.}^{131}\ (\%\ \text{of dose/ml.})}{\text{Ser.}^{127}\ (\mu\text{g./ml.})}$$

$$\text{II.} \qquad \text{Thy.}^{127}\ (\mu\text{g./min.}) = \frac{\text{Thy.}^{131}\ (\%\ \text{of dose/min.})}{\text{Ser.}^{131}\ (\%\ \text{of dose/ml.})} \times \text{Ser.}^{127}\ (\mu\text{g./ml.}).$$

The ratio on the right-hand side of equation II is the mean thyroidal clearance rate of serum iodide^{131} (ml./min.) in T; it can be determined exactly as outlined. The mean serum concentration of stable iodide in equation II is unknown and microchemically not determinable, because 1) the mass of serum iodide is too low, and 2) its precise separation from organically bound serum iodine is not possible.

However, salivary glands secrete and concentrate inorganic serum iodide as discussed later. It follows that the mean specific activity of iodide in mixed saliva is equal to the mean specific activity of iodide in serum at any given moment. Therefore, at the midpoint of the short time interval T:

$$\text{III.} \quad \frac{\text{Ser.}^{131}\ (\%\ \text{of dose/ml.})}{\text{Ser.}^{127}\ (\mu\text{g./ml.})} = \frac{\text{Sal.}^{131}\ (\%\ \text{of dose/ml.})}{\text{Sal.}^{127}\ (\mu\text{g./ml.})}$$

$$\text{IV.} \quad \text{Ser.}^{127}\ (\mu\text{g./ml.}) = \frac{\text{Ser.}^{131}\ (\%\ \text{of dose/ml.})}{\text{Specific activity of saliva iodide}\ (\%\ \text{of dose}/\mu\text{g.})}.$$

All values on the right-hand side of equation IV can be determined exactly. Thus the serum concentration of stable iodide has been evaluated. Putting this value into equation II, the thyroidal uptake rate of stable iodide may be computed.

RESULTS AND DISCUSSION

All values, determined in 16 subjects, are summarized in Table 1.

Method

Stanley (4) was the first to describe a method for the direct measurement of the thyroidal total iodine accumulation rate, using the equation: total thyroidal accumulation rate equals iodide^{131} uptake rate multiplied by the specific activity of the simultaneously excreted urinary iodine. Others used the same method (5–7), and their data agree well with those of Stanley as to thyroidal uptake rate and serum concentration of stable iodide.

All these calculations were based on the assumption that the specific activity of inorganic serum iodide equals the specific activity of total urinary iodine. However, there is evidence that the kidney excretes into urine not only inorganic serum iodide but also organic iodine compounds in small amounts (8–14). Also, some of the inorganic iodide in the urine is derived from thyroidal hormones and from other organic iodine compounds metabolized and deiodinated during their passage through the kidney (12, 15–18). Consequently, the specific activity of neither total urinary iodine nor inorganic urinary iodide equals the specific activity of inorganic serum iodide. The values for serum concentration and the thyroidal uptake rate of stable iodide, calculated by this method, would be too high. Moreover, a relatively long period for urine collection impairs the precise calculation of the specific activities, because the specific activity of serum iodide decreases rapidly with the time following the injection.

TABLE 1

Subject		Clinical state of thyroid function	BMR* (%)	Thyroidal			Serum concentration			Remarks	Previous treatment
No.	Name			Radioiodide accumulation rate** (% dose/1st hr.)	Clearance rate of serum iodide (ml./min.)	Uptake rate of stable iodide (μg./hr.)	Stable iodide (μg./ml.)	PBI† (μg./100 ml.)	PBI^{131}‡ (% dose/L.)		
1	H.M.	Hypothyroidism	− 4	20	36.0	0.155	0.000072	0.99	0.096	Anorexia nervosa	
2	K.B.	Hypothyroidism	−16	21	42.0	0.51	0.00020	1.12	0.235	Cancer of thyroid	
3	H.R.	Euthyroidism	+ 5	16.5	28.7	1.103	0.00064	6.24	—		
4	P.K.	Euthyroidism	+20	20	37.8	3.36	0.00148	6.05	0.088		
5	A.B.	Euthyroidism	− 1	27	47.9	8.16	0.00284	8.32	0.405		
6	I.S.	Euthyroidism	+ 2.5	26	52.6	2.46	0.00078	4.97	—	Nodular goiter	
7	E.W.	Euthyroidism	+53 (?)	21	50.7	11.4	0.00375	18.13	0.123	Endemic goiter	Stable iodine
8	J.A.	Euthyroidism	− 8.5	22	59.0	2.23	0.00363	4.54	0.326	Endemic goiter	
9	I.K.	Euthyroidism	+18	59	161.0	1.45	0.00015	3.49	0.835	Toxic goiter	$Radioiodine^{131}$
10	A.K.	Euthyroidism	+21	70	280.0	16.8	0.00100	5.67	1.53	with malignant exophthalmos	Propylthiouracil
11	H.E.	Hyperthyroidism	+40	79	620.0	3.72	0.00010	6.29	1.86		Propylthiouracil and methimazole
12	J.D.	Hyperthyroidism	+42	8.5	16.7	1.62	0.00161	9.44	0.008		$KClO_4$
13	E.H.	Hyperthyroidism	+44.5	28.5	59.4	14.70	0.00413	12.89	0.402		
14	H.S.	Hyperthyroidism	+14.5	41	104.6	10.10	0.00161	8.24	0.172		
15	G.R.	Hyperthyroidism	+41.5	82	651.0	25.4	0.00065	11.24	1.163		$Radioiodine^{131}$
16	A.W.	Hyperthyroidism	+60	85	1380.0	212.0	0.00256	>50.0	0.585		

* Normal range, −21 to +21 per cent.

** Normal range, 5 to 35 per cent of dose per first hour after injection.

† Normal range, 4 to 8 μg. per 100 ml.

‡ Normal range—values smaller than 0.4 per cent of dose per liter, forty-eight hours after injection.

Salivary glands

Salivary glands secrete and concentrate serum iodine (19–24). Furthermore, it has been shown that iodine in saliva is in the inorganic form (22, 25–29), even when large amounts of different organic iodine compounds have been administered previously (22, 25, 26, 28, 29). Therefore, the property of secreting and concentrating inorganic serum iodide might be attributed to the salivary glands. However, investigations have been carried out which point to the existence of organically bound iodine in saliva (30, 31), and suggest that the salivary glands may be involved in the deiodination of tyrosine and thyroxine in the body; thus some of the salivary iodide would be derived from the degradation of thyroidal hormones (32–34). These studies have not been confirmed by others (28, 35, 36). Moreover, we have demonstrated that the salivary glands secrete exclusively inorganic serum iodide, and that there is no secretion or deiodination of organic iodine compounds such as tyrosine or thyroxine by these glands (37). There is an extremely rapid transport of iodide between serum and saliva, with a negligible delay in the transit of iodide through the salivary glands (22–24, 37). It follows that the mean specific activity of iodide in mixed saliva (continuously secreted and withdrawn some minutes before each real collection period) equals the mean specific activity of inorganic serum iodide at any given moment. The specific activity of saliva iodide and especially the concentration of saliva iodide are determinable exactly, because the salivary glands concentrate inorganic serum iodide (23).

Serum concentration of stable iodide

Comparison of our data (Table 1) with those of others (4–7) shows a fair agreement, except that our values are much smaller, as was to be expected theoretically. They ranged from 0.0072 to 0.413 μg. per 100 ml. of serum. The mean was 0.1388 μg., that is, 1.41 per cent of the mean for serum PBI or 1.39 per cent of the mean for total serum iodine (ranging from 0.16 to 3.28 per cent of the total serum iodine). There was no significant difference between the concentrations of serum iodide in subjects with various thyroid conditions. The serum concentration of stable iodide was fairly constant during the period of examination (two hours).

Thyroidal uptake rate of stable iodide

According to equation II, this rate depends upon:

1) The serum concentration of stable iodide, *i.e.*, an extrathyroidal factor. From the curve in Figure 1 it may be deduced that there is a linear relationship in the non-hyperthyroid range. Therefore, some fluctuations of the calculated thyroidal uptake rates of stable iodide might be expected as

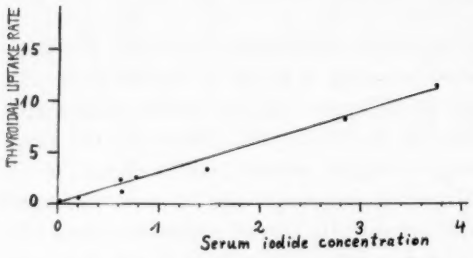

FIG. 1. Relationship of thyroidal uptake rate of stable iodide (μg. per hour) to serum iodide concentration (μg. per liter) in subjects not hyperthyroid and not previously treated with regard to the thyroid gland.

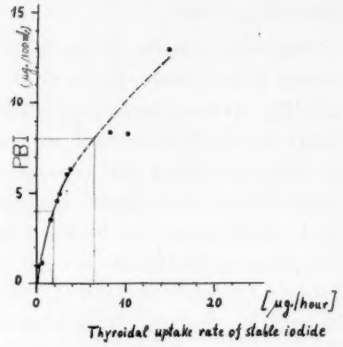

FIG. 2. Relationship of serum PBI level to thyroidal uptake rate of stable iodide as a measure for hormone formation and release in subjects previously untreated with regard to the thyroid gland. The normal range of PBI concentration is marked by fine lines, and the corresponding normal range of the thyroidal uptake rate of stable iodide may be deduced by the intersections of the lines with the curve.

a result of physiologic changes in the plasma iodide level following variations in dietary iodine.

2) The thyroidal iodide clearance rate, *i.e.*, a complex thyroidal factor which varies with the mass and activity of thyroid tissue, with the blood flow in the gland, and with intrathyroidal iodine metabolism. Thyroidal clearance seems to be fairly independent of variations in serum iodide concentration at low levels (4). Therefore, it is a reliable index of the capacity of the gland to accumulate stable iodide at any given concentration of serum iodide. The clearance values were observed to be fairly constant, at least for periods of two hours, in euthyroid subjects. In hyperthyroid subjects, within a relatively short time after injection, appreciable amounts of radioiodide taken up by the gland were being transformed and released into the plasma as hormonally bound iodine[131]; this was separated from serum iodide by the anion exchange resin Amberlite IRA 400. Consequently, radioactivity measured within the thyroid gland equals only the difference between accumulated and secreted radioiodine; hence, clearance values progressively decrease with the time after the injection. Therefore, the clearance rate should be determined as soon as possible after injection in order to permit the calculation of the real thyroidal uptake rate of stable iodide.

Hormone formation and release

Assuming that all of the stable iodide taken up by the thyroid gland is ultimately converted to hormonally active material, then the value for the thyroidal uptake rate of stable iodide may be taken as a quantitative meas-

ure of the formation and release of thyroid hormone; it would also be in good correlation with serum PBI level (Fig. 2). From the curve in Figure 2 a normal range of thyroidal uptake rate of stable iodide may be deduced; it ranges from 1.8 to 6.4 μg. per hour, or from 43.2 to 153.6 μg. per day. This is equivalent to a thyroidal secretion rate for *l*-thyroxine ranging from 66.6 to 237.0 μg. per day. In hyperthyroid Subject 13, the daily thyroidal secretion rate of iodine might be 352.8 μg., or 544 μg. of *l*-thyroxine. These values are in fair agreement with those calculated by other indirect methods (38–43).

Correlation between thyroidal radioiodide accumulation rate and uptake rate of stable iodide

Let T represent a 60-minute period from 0 to 60 minutes after injection of iodide^{131}. Then, from equation II, is derived equation V:

$$\text{V.} \qquad \text{Thy.}^{131}\ (\%\ \text{dose/hr.}) = \frac{\text{Thy.}^{127}\ (\mu\text{g./hr.}) \times \text{Ser.}^{131}\ (\%\ \text{dose/ml.})}{\text{Ser.}^{127}\ (\mu\text{g./ml.})}.$$

From Figure 3 it may be seen that the radioiodide accumulation rate (determined as the percentage of the dose in the first hour) cannot be taken as a direct indicator of uptake rate (μg. per hour) of stable iodide. This can be explained by the varying values for the specific activity of serum iodide in any subject. There may be a high radioiodide accumulation rate in spite of a low uptake rate of stable iodide (Subject 11), and vice versa (Subject 13). Assuming serum iodide concentration to be constant in all subjects—for example, 0.001 μg. per ml.—then the curve in Figure 3 would represent the relationship of the thyroidal radioiodide accumulation rate to this theoretically expected uptake rate of stable iodide calculated by means of equation II.

Correlation between thyroidal radioiodide accumulation rate and iodide clearance rate

According to equation V, the radioiodide accumulation rate depends upon:

1. The iodide clearance rate (Fig. 4). This is a fairly constant factor, which reflects the capacity of the total gland to accumulate stable iodide. It may be determined, too, by measurement of the radioiodide clearance rate.

2. The serum concentration of radioiodide. This is a changing value due to the change in dilution space with the time elapsed after injection of iodide^{131} (entrance of iodide^{131} into red blood cells, diffusion into the extracellular fluid space, penetration of the body cellular tissues and renal clearance of serum iodide^{131}). Assuming constancy of this time change in different subjects with various states of thyroid function, then the time course of serum iodide^{131} concentration would depend mainly upon the

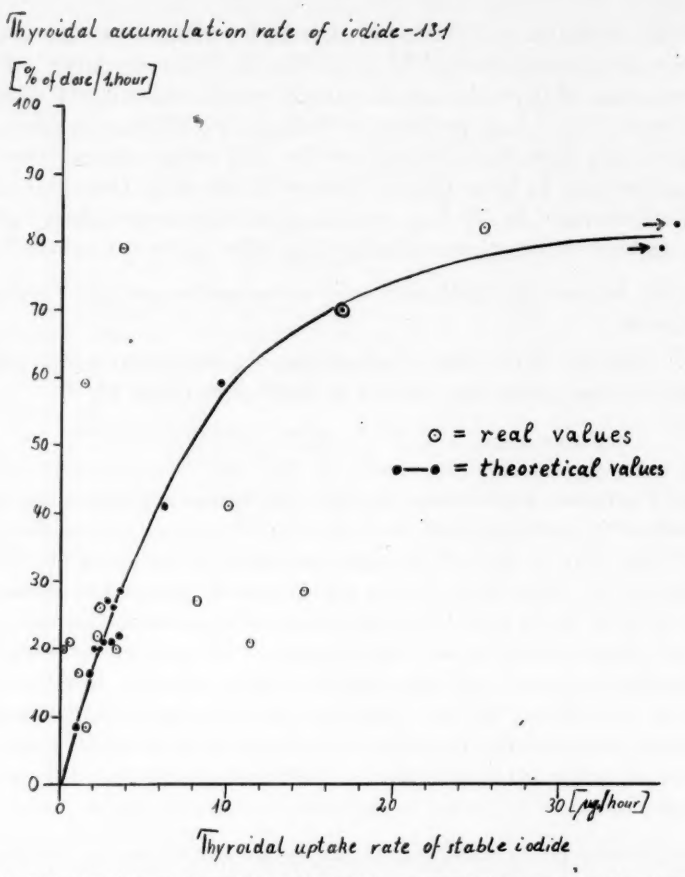

FIG. 3. Relationship of thyroidal radioiodide accumulation rate to the determined uptake rate of stable iodide (circles). The relationship which is to be expected theoretically, assuming serum iodide concentration to be constant (*e.g.*, 0.001 μg./ml.), is plotted by a curve related to the dots, which were calculated by means of equation II.

magnitude of the thyroidal clearance rate of serum iodide[131]. In hypothyroid subjects, serum radioiodide concentration would decrease slowly after the injection; in hyperthyroid subjects it would decrease rapidly. Therefore, with increasing thyroidal clearance values, the increase in the radioiodide accumulation rate (percentage of the dose in the first hour) has to become smaller. The course of the determined curve, representing this relationship, corresponds with this theoretical expectation (Fig. 4).

It follows that the radioiodide accumulation rate reflects merely the thyroidal clearance rate of iodide, but the clearance values are more reliable

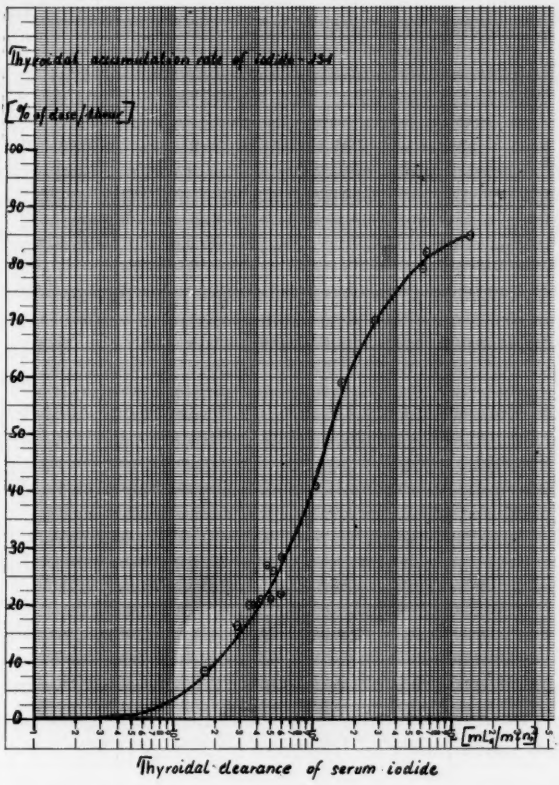

FIG. 4. Relationship of thyroidal radioiodide accumulation rate to iodide clearance rate. It may be seen that the radioiodide accumulation rate merely reflects the clearance rate of iodide. The course of the curve depends upon the decreasing values of serum radioiodide with increasing capacity of the gland to accumulate iodide.

for differentiation of the iodide-accumulating capacity or activity of the gland, especially in the hyperthyroid range (Fig. 4). However, neither the radioiodide accumulation rate nor the iodide clearance rate reflects the actual uptake rate of stable iodide.

Comparison of experimental results with the clinical state of thyroid function

The thyroidal radioiodide accumulation rate and the thyroidal clearance rate of serum iodide, in general, are in good agreement with the clinical state of thyroid function, especially in subjects previously untreated with regard to the thyroid gland (Subjects 3, 4, 6, 8, 13, 14, 15 and 16). However, there are subjects in whom these laboratory findings do not correspond with the clinical findings. The discrepancies may be explained by the

quantitative investigation of iodine metabolism. The results (Table 1) show that:

1. The radioiodide accumulation rate and the clearance rate may be normal, but the thyroidal uptake rate of stable iodide may be abnormally low or high. Therefore hormone formation and the serum PBI level may be low or high as a result respectively of a very low (Subjects 1 and 2) or a very high concentration of serum iodide (Subjects 5, 7 and 13).

2. The radioiodide accumulation rate and the clearance rate may be high, but the thyroidal uptake rate of stable iodide, the rate of hormone formation and the level of serum PBI may be normal or low as a result of very low concentration of serum iodide (Subjects 9 and 11).

3. The radioiodide accumulation rate, the clearance rate and the uptake rate of stable iodide may be high due to toxic goiter (Subject 10 and 11), but the serum PBI level may be in the normal range as a result of factors disturbing normal hormone formation by blocking the organic binding of accumulated iodide, as by propylthiouracil (Subject 10 and 11) or by methimazole (Subject 11).

4. In hyperthyroid subjects the radioiodide accumulation rate, the clearance rate and the uptake rate of stable iodide may be low in spite of a high concentration of serum iodide, as a result of factors blocking the iodide-trapping mechanism of the thyroid, as by KSCN or by $KClO_4$ (Subject 12).

5. Some subjects with a normal or a high radioiodide accumulation rate, clearance rate and uptake rate of stable iodide, may have hypothyroidism due to another disturbance of hormone synthesis (44).

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RENAL FUNCTION IN HUMAN PREGNANCY. III. EFFECTS OF ANTIDIURETIC HORMONE (ADH) ON RENAL HEMODYNAMICS AND WATER AND ELECTROLYTE EXCRETION NEAR TERM AND POST PARTUM*

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ABSTRACT

It has been suggested that pregnant women are less sensitive to the action of ADH because they possess an inactivating mechanism for this hormone. This hypothesis was tested by studying the effects of intravenous administration of Pitressin on renal hemodynamics and water and electrolyte excretion in pregnant women in the latter part of gestation and in postpartum and nonpregnant subjects. During pregnancy, a single intravenous dose of 100 milliunits (m.u.) of Pitressin or continuous infusion of this hormone in doses varying from 25 to 100 m.u. per hour produced a marked fall in urine flow with a simultaneous fall in renal plasma flow, glomerular filtration rate, and the output of sodium, chloride and total solute. Potassium excretion was inconsistent. Osmolal and free water clearances fell, the latter becoming negative in most instances. Following delivery or in the nonpregnant state, the same or higher doses of Pitressin produced a reduction in urine flow which was of the same magnitude and pattern as that in the pregnant subjects. However, renal hemodynamics remained unchanged and electrolyte excretion even increased. This response was typical of the action of Pitressin as described in the literature. Although various hypotheses are offered to explain the altered response to Pitressin in pregnancy, at present it is not possible to identify precisely the factors that account for the change in renal hemodynamics and the fall in solute excretion. The results of the present study do not lend support to the hypothesis suggested by others that an inactivating mechanism for ADH exists in human pregnancy.

SEVERAL reports have claimed that extracts of plasma or placenta obtained from normal pregnant women inactivate antidiuretic hormone (ADH) *in vitro* (1–4). These studies have implied that pregnant women

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should respond less than nonpregnant subjects to the action of ADH. This implication has received some support from isolated reports which indicate that patients with diabetes insipidus become worse and require more antidiuretic hormone (Pitressin) during pregnancy than after delivery (5, 6).

Recent observations from our laboratory have shown that the antidiuresis and antinatriuresis associated with venous pooling in pregnant women become more pronounced as pregnancy progresses, reaching a maximum in the latter part of gestation (7). Since one of the mechanisms of the oliguria induced by venous pooling is said to be excessive secretion of ADH, the marked renal response speaks against inactivation of this hormone in pregnant subjects.

The present studies were aimed at further elucidating this problem by investigating the effects of exogenously administered ADH on renal hemodynamics and water and electrolyte excretion in the latter part of gestation and after delivery. It was hoped that these studies might furnish information regarding the response of pregnant subjects to ADH and the possible existence of an inactivating mechanism for this hormone during normal gestation.

MATERIAL AND METHODS

The studies were performed on 11 normal pregnant women whose ages varied from 20 to 37 years and whose gestation varied from 34 to 40 weeks. Two of the women were studied again six to eight weeks post partum, and their data together with those obtained from 3 healthy nonpregnant women constitute the nonpregnant control. The test was carried out in the hospital in the postabsorptive state in a quiet room with the patient lying in bed in the supine or in the lateral recumbent position. Induction of water diuresis, administration of inulin and para-aminohippurate (PAH) and collection of blood and urine specimens followed techniques previously described (7).

After forty to sixty minutes of equilibration time, 3 to 4 thirty-minute control clearances were obtained. Thereafter, Pitressin[1] was injected intravenously and 3 to 8 clearances of twenty to thirty minutes' duration each were obtained. In 6 subjects tested during pregnancy and 1 tested post partum a single dose of 100 milliunits (m.u.) of Pitressin was given intravenously over a period of one to two minutes. In the 5 remaining subjects, intravenous priming injections of Pitressin varying from 25 to 100 m.u. were administered and were followed by a constant infusion adjusted so as to deliver between 25 and 100 m.u. per hour for the duration of the study.

Assays of inulin, PAH, sodium, potassium and chloride in blood and urine as well as estimation of renal plasma flow, glomerular filtration rate, total solute excretion, and osmolal and free water clearances followed techniques described elsewhere (7).

RESULTS

Systemic effects

In the doses given, intravenous Pitressin did not produce any striking systemic side-effects. Transitory pretibial edema of varying degrees occurred in the majority of the patients who received the drug by continuous

[1] Aqueous solution of Pitressin was furnished by Parke, Davis & Company from a single fresh lot No. 3-184-4. The same lot was used in all the patients.

drip infusion. Blood pressure and pulse rate remained unchanged. No perceptible uterine contractions occurred in the pregnant subjects after either the single injection or the constant infusion of Pitressin.

Renal effects in pregnancy

In Table 1 the data on the effects of Pitressin in pregnancy are presented. Listed for each subject are the averages for the control periods and the values for individual clearances obtained after injection of Pitressin. Figure 1 illustrates a typical example of the response of a pregnant subject to continuous administration of Pitressin.

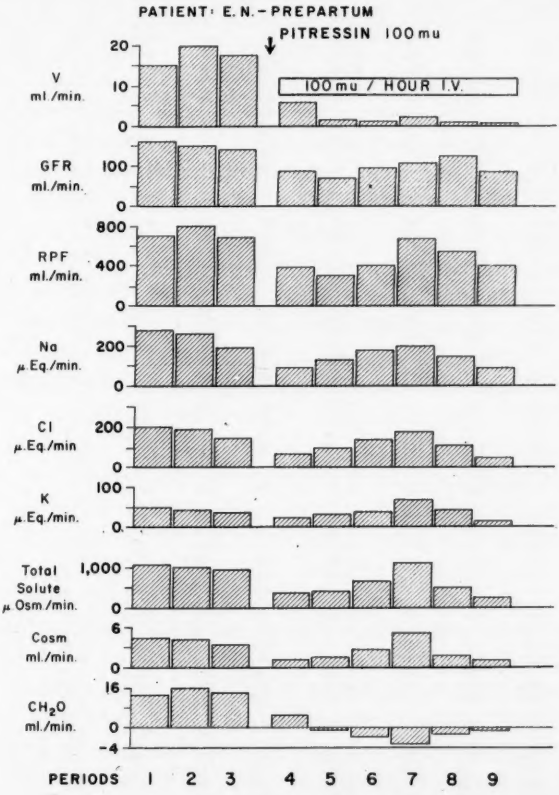

FIG. 1. Changes in urine flow (V), glomerular filtration rate (GFR), renal plasma flow (RPF), the excretion of Na, Cl, K and total solute and the osmolal (Cosm) and free water clearance (CH_2O) induced by the intravenous injection of a priming dose of 100 m.u. of Pitressin followed by a constant infusion of 100 m.u. per hour, in a pregnant subject. The fall in urine flow was concomitant with a fall in GFR, RFP and in the excretion of electrolytes and total solute. There was also a marked fall in free water clearance.

TABLE 1. DATA ON THE EFFECTS OF PITRESSIN IN 11 PREGNANT WOMEN

The values listed represent urine flow (V), renal plasma flow (RPF), glomerular filtration rate (GFR), excretion of Na, K, Cl and solutes, and osmolal (Cosm) and free water (CH_2O) clearances. Pitressin was given in a single injection or in a priming dose followed by a constant infusion. The values above the top horizontal line in each section represent the average for the control periods and those below the line represent the individual clearances after administration of Pitressin.

Patient	V (ml./ min.)	RPF (ml./ min.)	GFR (ml./ min.)	Na (μEq./ min.)	$\frac{Na^*}{P \times GFR}$	K (μEq./ min.)	Cl (μEq./ min.)	Total Solute (μOsm/ min.)	Cosm (ml./ min.)	CH_2O (ml./ min.)	Dose of Pitressin, intravenously
	16.3	750	154	216	1.05	42	46	700	2.8	13.6	
B.J.	3.6	415	92	160	1.97	40	30	400	—	—	
	3.4	314	57	158	1.90	37	21	200	1.0	2.4	100 m.u., single inj.
	2.6	727	144	71	0.35	87	77	655	2.4	0.2	
	9.6	1333	235	220	0.66	58	134	1148	4.1	5.5	
	10.0	547	103	331	2.14	130	218	1580	5.7	4.3	
B.M.	4.3	227	51	125	1.75	35	162	509	1.9	2.4	
	4.1	265	58	83	1.00	34	37	402	1.5	2.6	100 m.u., single inj.
	14.7	715	135	394	2.95	130	174	1652	5.9	8.8	
	12.0	960	176	164	0.70	48	44	700	3.0	9.0	
M.P.	3.0	448	95	95	0.80	30	32	363	1.9	1.1	
	2.3	348	61	73	0.52	20	30	263	0.9	1.4	100 m.u., single inj.
	2.0	508	95	173	1.21	34	94	548	2.0	0.0	
	10.7	1464	246	299	0.82	80	118	1156	4.4	6.3	
	15.4	1155	215	148	0.39	110	77	957	3.5	11.9	
L.S.	2.5	390	84	49	0.41	27	29	236	0.9	1.6	
	1.2	362	72	12	0.12	33	37	296	1.1	0.1	100 m.u., single inj.
	7.1	1337	246	98	0.30	121	64	871	3.3	3.8	
	11.4	710	146	115	0.65	58	59	598	2.3	9.1	
B.D.	2.3	462	81	57	0.50	33	39	268	1.0	1.3	
	1.0	467	87	106	0.55	54	83	405	1.7	−0.7	100 m.u., single inj.
	1.4	757	128	158	0.88	58	112	605	2.3	−0.9	
	5.7	856	189	186	0.71	84	126	963	3.6	2.1	
	12.3	654	167	214	1.09	146	41	1223	4.3	8.0	
A.H.	6.0	352	81	75	0.66	62	15	522	1.8	4.2	
	1.8	245	62	23	0.69	60	8	426	0.6	1.2	
	2.8	242	63	68	0.78	61	24	426	1.6	1.2	100 m.u., single inj.
	5.2	635	159	161	0.73	83	68	1000	3.7	1.5	
	8.6	904	256	247	0.70	238	115	1673	5.9	2.7	
	17.0	735	151	242	1.22	43	175	1003	4.0	13.0	
E.N.	6.6	332	82	76	0.67	26	57	313	1.3	5.3	
	1.3	384	70	117	1.20	34	94	367	1.5	−0.2	
	1.2	424	96	200	1.52	43	155	727	2.9	−1.7	100 m.u. plus
	1.3	674	110	185	1.49	40	184	413	4.9	−3.6	100 m.u./hr.
	0.7	554	133	161	0.89	44	115	451	1.9	−0.8	
	0.4	397	93	76	0.60	18	50	238	1.0	−0.6	
	18.8	920	185	117	0.48	80	154	1150	4.3	14.5	
M.M.	5.8	1078	142	242	0.72	163	137	1517	5.9	−0.1	
	2.5	872	189	232	0.90	84	164	1245	4.8	−2.3	
	2.3	821	186	273	1.08	68	193	1340	5.0	−2.7	50 m.u. plus
	2.0	888	193	245	0.94	66	175	1229	4.7	−2.7	50 m.u./hr.
	1.8	830	189	214	0.85	76	174	1156	4.3	−2.5	
	13.8	1322	236	272	0.71	68	126	1148	4.2	9.6	
C.C.	7.9	770	137	197	1.04	43	117	684	2.4	5.5	
	2.1	655	85	149	1.24	30	108	445	1.7	0.4	
	2.5	669	118	223	1.35	42	170	673	2.6	−0.1	50 m.u. plus
	2.8	923	153	222	1.50	193	140	922	3.5	−0.7	50 m.u./hr.
	1.5	498	86	235	1.99	164	188	618	2.4	−0.9	

* $\frac{Na}{P \times GFR}$ = sodium excretion expressed as a fraction of the filtered load.

TABLE 1.—*Continued*

Patient	V (ml./ min.)	RPF (ml./ min.)	GFR (ml./ min.)	Na (μEq./ min.)	$\frac{Na^*}{P \times GFR}$	K (μEq./ min.)	Cl (μEq./ min.)	Total Solute (μOsm/ min.)	Cosm (ml/ min.)	CH_2O (ml./ min.)	Dose of Pitressin, intravenously
	17.2	704	169	250	1.30	94	129	1416	5.2	12.0	
S.M.	4.9	277	78	99	0.92	22	45	536	1.9	3.0	
	1.4	201	77	95	0.91	18	51	434	1.6	−0.2	
	1.3	235	78	106	1.00	18	55	478	1.7	−0.4	
	1.2	225	76	93	0.94	18	48	445	1.6	−0.4	25 m.u. plus
	2.1	424	136	202	1.10	36	94	890	3.1	−1.0	25 m.u./hr.
	1.5	340	107	143	1.03	30	67	674	2.4	−0.9	
	0.7	234	76	98	1.00	14	43	433	1.4	−0.7	
	8.7	586	138	233	1.20	22	172	764	2.3	6.4	
C.W.	3.8	537	99	231	0.78	19	166	722	2.2	1.6	
	3.7	394	118	217	0.92	17	149	629	1.9	1.8	25 m.u. plus
	2.3	406	106	190	0.85	18	137	564	1.7	0.6	25 m.u./hr.
	3.2	487	113	218	0.99	23	165	727	2.2	1.0	

A marked water diuresis was obtained in all the patients as manifested by the average control urine flows which varied from 8.7 to 18.8 ml. per minute. The administration of 100 m.u. of Pitressin induced a fall in the urine flow which varied from 50 to 90 per cent of the control values. The decrease was evident within the first twenty minutes after the injection but became much more striking in the following forty to sixty minutes. Thereafter, the urine flow began to return toward control values. Renal plasma flow and glomerular filtration rate decreased in every subject in the first, second and occasionally in the third collection period after injection of Pitressin and returned to control values or higher in the last period (Table 1). The filtration fraction changed insignificantly. The excretion of sodium, chloride and total solute, expressed in absolute values (μEq. or μOsm per minute) decreased in every instance in the first 2 to 3 clearances after administration of Pitressin and returned to control values in the last clearance (Table 1). When expressed as a fraction of the filtered load, the excretion of sodium showed variation, increasing in some patients and decreasing in others after injection of Pitressin. Potassium excretion changed inconsistently. Osmolal and free water clearances fell consistently, the latter approaching zero or becoming negative in several instances. Both clearances returned to control values in the last period. Plasma levels of sodium, chloride, potassium, inulin and PAH remained practically unchanged.

Continuous intravenous infusion of Pitressin resulted in an even greater fall in urine flow, glomerular filtration rate and renal plasma flow (Table 1 and Fig. 1). The excretion of sodium, chloride and total solute fell markedly in all subjects except one (M.M.) who showed a slight fall in GFR and RPF but no significant change in the output of electrolytes (Table 1). Free water clearance was considerably reduced and became negative in nearly all instances (Table 1 and Fig. 1). All of these changes became more strik-

TABLE 2. DATA ON THE EFFECTS OF INTRAVENOUS PITRESSIN IN 3 NONPREGNANT AND 2 POSTPARTUM SUBJECTS

A priming dose of 100 m.u. was given and was followed by a constant infusion of 100 m.u. per hour (Patient A.H. had a single injection of 100 m.u.). Values above the top horizontal line in each section represent the average for the control periods, and those below the line represent the individual clearances after Pitressin.

The symbols are the same as in Table 1.

Patient	V (ml./min.)	RPF (ml./min.)	GFR (ml./min.)	NA (μEq./min.)	K (μEq./min.)	Cl (μEq./min.)	Total solute (μOsm/min.)	Cosm (ml./min.)	CH_2O (ml./min.)
	6.5	405	135	95	22	44	419	1.5	5.0
S.D.	1.3	169	58	69	11	29	184	0.7	0.6
	0.5	389	123	82	—	64	336	1.2	−0.7
nonpregnant	0.4	398	137	126	—	—	421	1.6	−1.1
	0.5	384	104	112	—	52	396	1.4	−0.9
	8.3	616	109	174	50	153	780	2.9	3.4
B.L.	1.8	582	117	195	43	219	771	2.9	−1.0
	1.1	606	105	233	56	203	758	2.8	−1.7
nonpregnant	0.9	657	119	214	21	173	707	2.5	−1.6
	1.1	691	145	256	14	194	796	2.9	−1.8
	10.7	555	120	98	102	85	711	2.8	8.0
D.J.	3.3	355	96	76	78	73	466	1.8	1.5
	0.8	501	111	103	85	94	510	1.9	−1.2
nonpregnant	1.2	594	169	182	105	148	838	3.2	−2.0
	1.2	567	153	183	107	159	814	3.1	−1.8
	14.0	679	137	257	104	194	1125	5.9	8.1
A.H.	6.8	405	84	202	45	175	711	2.5	4.2
	1.3	694	122	210	55	182	806	2.9	−1.6
post partum	1.9	655	140	315	55	250	1183	4.3	−2.4
	4.7	637	131	247	45	190	1030	3.7	0.9
	13.8	489	97	349	29	266	882	3.6	10.2
E.N.	8.4	425	99	331	43	276	870	3.4	5.0
	1.5	440	97	299	57	276	1096	4.2	−2.6
post partum	2.2	540	138	459	49	406	1344	5.3	−3.1
	2.3	469	99	476	44	418	1354	5.8	−3.5

ing during the second clearance after injection of Pitressin, and lasted for the duration of the infusion. It was not possible to detect any significant difference between the effects of the various doses employed.

Renal effects in nonpregnant subjects

In Table 2 are listed data on the effects of intravenous administration of Pitressin in nonpregnant and postpartum subjects. The average values for the control periods as well as the values for individual clearances following the injection of Pitressin are given. Figure 2 illustrates a typical response in a nonpregnant subject in the postpartum period.

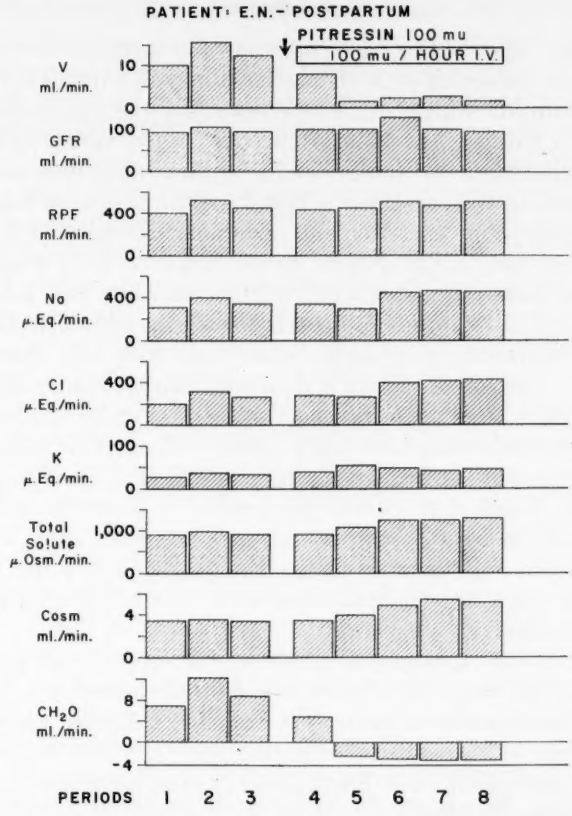

FIG. 2. Effects of intravenous Pitressin in the same patient presented in Figure 1, six weeks after delivery. The magnitude of the fall in urine flow and in free water clearance was the same as that in Figure 1. However, renal hemodynamics did not change significantly and the excretion of electrolytes and total solute increased. This response to Pitressin is typical of the nonpregnant state as described in the literature.

The urine flow fell markedly during administration of Pitressin and the fall was of the same pattern, magnitude and duration as that in the pregnant subjects (Table 2 and Fig. 2). Renal plasma flow and glomerular filtration rate, however, did not change significantly in any of the cases studied except in the first period after administration of Pitressin. Similarly, the excretion of sodium, potassium, chloride and total solute either remained the same or increased slightly. Osmolal clearance changed inconsistently, but the free water clearance decreased markedly and followed a trend similar to that in the pregnant subjects (Table 2 and Fig. 2). These results were consistent with those of the great majority of reports on the action of Pitressin in normal nonpregnant subjects (8–11).

DISCUSSION

It is not the object of this paper to review the enormous literature which has dealt with the action of ADH on the kidney in animals and man. Despite the confusion which still exists regarding the exact mechanism of action of this hormone, particularly in view of the variation in response from one animal species to another and according to the dose and the route of administration, the consensus is that Pitressin decreases the urine flow without altering solute excretion and renal hemodynamics (8–11).

A few exceptions to this general belief, however, have been observed. Barclay and Cooke (12) noted a fall in glomerular filtration rate and renal plasma flow in 8 of 13 normal subjects following the administration of Pituitrin (posterior pituitary extract). Nelson and Welt (13) observed a decrease in the excretion of sodium and chloride concomitantly with a reduction in urine flow after the intravenous or intramuscular administration of Pitressin to normal subjects and to patients with cirrhosis and ascites. Although these authors stated in their discussion that the decrease was minimal, analysis of their data shows that in many instances of cirrhosis of the liver the reduction exceeded 50 per cent of the control values. The authors did not measure renal hemodynamics and therefore it is not possible to state whether the fall in electrolyte excretion was in any way related to a fall in the glomerular filtration rate.

In a similar study also conducted on normal subjects and patients with liver diseases, Bernstein and his co-workers (14) observed a reduction in creatinine clearance and in electrolyte excretion following the administration of nicotine or Pitressin to cirrhotic patients. These authors thought that the fall might have been due to errors in urine collection caused by the rapid changes in urine flow.

Our data obtained on normal pregnant women showed that, in every instance, the administration of Pitressin in doses similar to those used in nonpregnant subjects resulted in a decrease in urine flow, glomerular filtration rate and renal plasma flow. The excretion of sodium, chloride and total solute was similarly reduced except in 1 subject. The changes occurred regardless of whether Pitressin was given in a single injection or by constant infusion and irrespective of the dose employed. These results were entirely different from those observed in the same subjects after delivery or in nonpregnant women, despite the fact that the procedure for the study was the same and the dose of Pitressin used was higher. Since, in most instances, the changes in electrolyte and solute excretion followed very closely those of the glomerular filtration rate, it is most likely that the decrease observed in pregnancy was due to a fall in the filtered load.

Several hypotheses may be offered to explain the unusual response of the pregnant woman to Pitressin:

1. *Increased activity of the autonomic control of the vascular tone.* In pregnancy, the vascular tone is largely sustained by an increased activity of the autonomic vasoconstrictor impulses (15–17). This increased activity represents a compensatory mechanism for the tendency to venous pooling caused by the pressure of the enlarging uterus on the major vessels and by the increased intra-abdominal and venous pressures. It is possible that these factors may alter the action of Pitressin on the kidney and may render the renal vessels susceptible to its effect. Under these circumstances, the change in renal hemodynamics may lead to a fall in solute excretion. This hypothesis receives some support from the fact that patients with cirrhosis and ascites, who also have increased intra-abdominal and venous pressures, usually respond to ADH in a manner similar to that of pregnant women.

2. *Decrease in cardiac output.* In animals, Pitressin in doses of 20 to 40 units may decrease cardiac force and cardiac output and increase vascular resistance (18). Renal vascular resistance may also increase, thus reducing renal blood flow, glomerular filtration and solute output. This possibility seems unlikely, since the dose of Pitressin required to produce such a myocardial effect is extremely large, whereas the dose used in our studies was comparatively small and was of the same magnitude as those used by us and by a number of other authors in nonpregnant subjects.

3. *Elevated blood levels of adrenocortical hormones and of oxytocin.* It is well known that, in pregnancy, there is a progressive increase in the secretion of both the mineralocorticoids and glucocorticoids which reaches its maximum in the latter part of gestation. Oxytocin is presumably also increased in the latter part of gestation. Gaunt and his co-workers (19) have indicated that, despite the complexity of the interrelationship between the adrenal cortex and the posterior pituitary, adrenal steroids may modify the action of ADH. For instance, the presence of high levels of aldosterone may reverse the effect of Pitressin on the excretion of electrolytes. Brooks and Pickford (20) have shown that, in dogs, the administration of oxytocin together with Pitressin may alter the effect of the latter on renal hemodynamics and electrolyte excretion and that the direction of the alteration depends to a large degree on the dose and the status of hydration of the animal. It is possible, therefore, that the elevated levels of corticosteroids in pregnancy, particularly of aldosterone, together with the presence of oxytocin may alter the effect of Pitressin on the kidney and may account for some of the changes observed in pregnancy.

4. *Rapid and marked changes in urine flow.* The decrease in renal plasma flow, glomerular filtration rate and solute excretion may be related to the rapid fall in urine flow caused by Pitressin. It has been stated that, except in extreme oliguria, the glomerular filtration rate does not depend to any great extent on the rate of urine flow (21). However, Ek (22) has shown

that marked diuresis may increase the renal plasma flow and glomerular filtration rate and extreme reduction of urine flow may be expected to lower them. Recently Levinsky and Berliner (23) demonstrated that the composition of the urine in the ureter and bladder may change considerably during periods of low urine flow because of movement of water and solutes. Under these circumstances, the urine collected from the bladder may not reflect the composition of the urine as it leaves the kidney. This situation is aggravated in pregnancy by the enlargement of the urinary tract "dead space" caused by the diminished tonus and by the dilatation of the renal pelvis and ureter. These factors, together with the displacement of the bladder imposed by the presenting part, may introduce errors in urine collections, particularly at low rates of urine flow, and therefore may vitiate the clearance values.

Although the low urine flow and the enlarged "dead space" probably contributed to the change in renal hemodynamics and in electrolyte excretion during the first and possibly the second clearance after administration of Pitressin, they can hardly explain the low values observed during prolonged infusion of Pitressin when the effects of incomplete mixing had presumably subsided. Furthermore, the magnitude of fall in urine flow was practically the same in the pregnant and nonpregnant subjects, so this factor cannot be the sole cause. The only other explanation is that the greatly enlarged "dead space" in pregnancy considerably increases the trapping of urine and somehow favors the movement of water and solutes beyond that observed in the nonpregnant state. Hence, errors in estimating renal clearances at a low level of urine flow in pregnancy are magnified.

The present studies contradict the hypothesis suggested by others that pregnant women are less sensitive to the action of Pitressin (24, 25) or that their plasma inactivates ADH. This notion has been based largely on somewhat uncontrolled studies in which a dose of Pitressin was injected into pregnant women and the concentration of sodium chloride in the urine was determined for a period of thirty to sixty minutes. Control values before the injection were not determined and the concentration of electrolytes was not related to the change in urine flow. Furthermore, the response of pregnant women was compared to that of patients with toxemia of pregnancy and not to that of nonpregnant subjects (24, 25).

The few observations (5, 6) to the effect that pregnant subjects with diabetes insipidus become worse and require more Pitressin during pregnancy than after delivery do not prove that the plasma in pregnancy inactivates ADH. Furthermore, these observations are contradicted by those of other investigators who have noted no change or even improvement in diabetes insipidus during pregnancy (26–30). Therefore, at present, it is not

possible to draw definite conclusions regarding the fate of ADH from the few observations on the course of diabetes insipidus in pregnancy.

The validity of the studies on the inactivation of ADH *in vitro* by the plasma of pregnant subjects or by placental tissue has also been questioned recently by Heller (31) on the basis that the posterior pituitary principles can be inactivated by several mammalian enzymes. In addition, this author has pointed out that the mere fact that a substance is inactivated *in vitro* by plasma or tissue can hardly apply to the intact organism. Until better methods are available for the study of the fate and metabolism of ADH, it is necessary to be cautious in applying *in vitro* results to human subjects.

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ASSESSMENT OF THE FUNCTIONAL CAPACITY OF THE ADRENAL CORTEX. II. CLINICAL APPLICATION OF THE ACTH TEST*

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ABSTRACT

In order to verify its clinical usefulness, the ACTH test was performed in a series of 34 proved cases of adrenocortical hyperfunction and hypofunction. The results were above the previously established upper fiducial limit of normal response in the cases of hyperfunction, and below the lower limit in the cases of hypofunction. The present study confirms the findings of earlier investigators, that adrenocortical adenoma and adrenocortical hyperplasia with Cushing's syndrome exhibit different reaction patterns to stimulation with ACTH. In addition, the existence of patients with a normal resting excretion of corticosteroids but complete lack of adrenocortical reserve capacity was confirmed. Sixty-six patients with at least one of the following six classic symptoms of Addison's disease (loss of weight, weakness, hyperpigmentation, hypotonia, hypoglycemia, and disturbance in electrolyte and/or water metabolism) were studied clinically and with the aid of the ACTH test. The clinical investigation excluded other debilitating disease as a possible cause of the symptom(s). In 43 of the 66 patients there was a pathologically low urinary corticosteroid response to stimulation with ACTH. In approximately half of these cases there was also a pathologically low pretreatment resting excretion of corticosteroids. Almost without exception, the patients showing a normal response to ACTH had only one clinical symptom, namely, weakness. About 80 per cent of the patients with two or more symptoms showed a pathologically low excretion of corticosteroids in response to ACTH. The results indicate that the previously described method for the assessment of adrenocortical reserve capacity makes clinical sense.

IN A previous paper (1) we described an improved method for assessing the functional reserve capacity of the adrenal cortex. A highly significant correlation was found between the logarithms of urinary levels of 17-hydroxycorticosteroids during rest and after stimulation with adrenocor-

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ticotropin (ACTH). On the basis of this correlation an attempt was made to establish a "normal range" for clinical ACTH test results, within which could be expected to lie the reactions in 95 per cent of healthy subjects.

As the next step it was necessary to find out whether or not this test made clinical sense. Several methods are possible for an analysis of this type. The best would seem to be study of well documented cases of adrenocortical hyperfunction or hypofunction, in order to see if their response to the ACTH test is, respectively, above or below the range established from the earlier described normal material. In the selection of cases the main difficulties lie in clinically demonstrating with objective criteria the presence of hyperfunctlon or hypofunction. If this can be accomplished, the ACTH test can be evaluated with a fairly high degree of safety.

As regards adrenocortical hyperfunction in the cases now to be presented, histologic examination of adrenal tissue could confirm the underlying cause of the characteristic symptoms which led to selection for the ACTH test. In a smaller group of patients with hypofunction—for instance, following adrenalectomy or due to chromophobic pituitary adenoma—the situation was similarly favorable. In most of the patients with hypofunction the sum of the signs and symptoms characteristic of adrenocortical hypofunction was accepted, in accordance with general clinical practice, as an indicator of the functional status of the adrenal cortex.

We based our evaluation of such manifestations on the findings described by Thorn and Jenkins (2) in 125 cases of Addison's disease. Predominant in these patients were weakness (in 99 per cent of the series), pigmentation (98 per cent), weight loss (97 per cent), diffuse gastro-intestinal symptoms (90 per cent) and hypotension (87 per cent). Of the other symptoms, none was present in more than 35 per cent of the patients. For reasons which will be discussed later, the gastro-intestinal symptoms in our patients are not reported in detail. We considered primarily the other four dominant manifestations of Addison's disease. In addition, we recorded disturbances in carbohydrate metabolism and in electrolyte or water balance, since these can readily be demonstrated objectively. On this basis we analyzed a series of patients in whom the diagnosis of Addison's disease was regarded as well established according to the foregoing criteria.

In addition, ACTH tests were carried out on a group of patients who had one or more of the manifestations characteristic of adrenocortical hypofunction and no definite clinical signs of other disease.

This paper thus reports the results of ACTH tests in approximately 100 patients with manifest or suspected hyperfunction or hypofunction of the adrenal cortex.

CLINICAL MATERIAL

I. *Hyperfunction of the adrenal cortex (13 patients)*

In all the patients with adrenocortical hyperplasia (8 cases), adrenocortical adenoma (4 cases) or cancer of the adrenal (1 case), the diagnosis was verified by histologic study of surgically removed specimens. The clinical manifestations, except in the patient with cancer and in 2 with the adrenogenital syndrome, were characteristic of Cushing's syndrome. None of the patients had clinical or other signs of pituitary tumor.

IIa. *Clinically established hypofunction of the adrenal cortex (21 patients)*

In 3 cases of bilateral adrenalectomy for metastasizing cancer of the breast, the ACTH test was performed during continuous daily administration of 50 mg. of cortisone. The diagnosis of hypopituitarism was verified by clinical signs and laboratory tests (using conventional methods) of concomitant hypofunction of the thyroid, adrenals and ovaries or testes; 3 of the 7 patients with hypopituitarism had chromophobic pituitary adenoma, which was subsequently confirmed when the gland was excised. In the 11 cases of Addison's disease the diagnosis was made clinically according to generally accepted criteria. The main symptoms were *loss of weight, weakness with reduced fitness for work, hyperpigmentation, hypotension, hypoglycemia and disturbed electrolyte and/or water balance.* The first four manifestations were present in all the patients, and the last two manifestations in all but 2 patients who were receiving exogenous cortisone. Other symptoms of this disease, such as anorexia, vomiting and intestinal distress, were present in many instances, but as these clinically well known manifestations scarcely are relevant to the following discussion, they are not presented in detail. Clinical conditions with superficial resemblance to Addison's disease, such as disorders of the small intestine, were not included.

IIb. *Clinically suspected hypofunction of the adrenal cortex (66 patients)*

The ACTH test was also performed on 66 patients who had at least one of the following six classic symptoms of Addison's disease—*weight loss, weakness, hyperpigmentation, hypotension, disturbed carbohydrate metabolism and water imbalance.* It was thought that the ACTH test might reveal partial reduction of adrenocortical function in some of these patients. This group was collected over a three-year period. Some of the patients were primarily hospitalized at Karolinska Sjukhuset, and others were selected following steroid determinations on urine specimens sent to the Hormone Laboratory. If one or more of the foregoing clinical manifestations was present, an ACTH test was performed and the patient was clinically investigated according to the criteria stated subsequently. Since cases may be referred to Karolinska Sjukhuset from the whole of Sweden and since this Hormone Laboratory serves about 70 hospitals in the central and northern regions of the country and also accepts specimens for analysis from southern Sweden, the underlying population upon which the case material is based can be estimated to be at least 2 or 3 million people. This may explain the relatively great number of cases with adrenocortical dysfunctions reported in this study. Forty of the patients were clinically examined at Karolinska Sjukhuset, and the remaining 26 were examined at other hospitals in central and northern Sweden.

Weakness was the symptom which most commonly prompted investigation. Frequently weakness was so great that it gravely affected the working capacity. In the patients examined at Karolinska Sjukhuset, work tests, according to the method of

TABLE 1. DISTRIBUTION OF MAIN SYMPTOMS IN 66 PATIENTS WITH SUSPECTED ADRENOCORTICAL HYPOFUNCTION

	Weight loss	Weakness	Pigmentation	Hypotension	Hypoglycemia	Disturbed water balance
No. of pathologic cases/No. of cases	16/66	63/66	6/66	25/66	4/45	2/41

Wahlund (3) were performed at the Physiologic Clinic. Invariably there was reduction of physical power according to the established normal values for the age and sex. By loss of weight was meant a decrease of 5 to 12 Kg. within eighteen months. Hyperpigmentation was considered to be present when the color and location of the areas were characteristic of, or suggestive of Addison's disease. One case in which hyperpigmentation was confined to yellowish discoloration of the forehead was not included. Hypotension was defined as morning blood pressure of 105/65 mm. Hg or less. Carbohydrate metabolism was studied on the basis of the fasting blood sugar level and the results of oral glucose tolerance tests. The concentration of sodium and potassium was determined in the serum and, in a limited number of cases, also in the urine. Tests of water balance were made according to the procedure of Luft and Sjögren (5), whose criteria were used in judging the results.

The diagnoses for which the 66 patients were first referred for hospital investigation were asthenia, hypotension, orthostatic anemia or suspected endocrine disorders. Vasoregulatory asthenia according to the definition of Holmgren *et al.* (6) was confirmed in 14 cases and was suspected in 2 others; one of the main components of this syndrome is weakness with obviously impaired physical power. Chronic debilitating diseases entailing weight loss and weakness, such as cancer, were excluded from the series. When clinical examination clearly revealed other pathologic conditions, the case was likewise excluded. Chronic disease of the small intestine, which in some of its features may resemble the cases under discussion, is an example of such conditions. The results of investigations in a group of cases of this type will be separately presented at a later date. The distribution of the clinical symptoms is shown in Table 1.

A detailed account of the clinical symptoms is presented in association with the results of the ACTH tests. In 15 patients the basal metabolic rate ranged from −5 to −20 per cent, but the serum protein-bound iodine level and the thyroidal uptake of I^{131} were normal. In the other cases in which thyroid function could be satisfactorily studied, these parameters and the basal metabolic rate were normal. The concentration of sodium and potassium in the serum was within normal limits in all cases. In the 10 cases in which the urinary content of these cations was studied, the findings were likewise normal.

Analytical methods

For a full description of the analytical methods employed in this study the reader is referred to our previous report (1). Briefly, urinary 17-hydroxycorticosteroids (17-OH-CS) were measured with a modification of the technique described by Appleby *et al.* (7), and 17-ketosteroids (17-KS) were estimated with the micromethod of Vestergaard (8), using the color correction equation of Allen (9). For separate estimation of

dehydroepiandrosterone, androsterone+etiocholanolone and 11-oxygenated 17-ketosteroids, chromatographic separation was performed on the ketonic fraction following hydrolysis with sulphuric acid, as described in detail previously (10).

Performance of the ACTH test

A detailed description of the technique has already been given (1). After two 24-hour urine specimens (resting level) had been collected, a dose of 25 I.U. of a commercial preparation of ACTH (Acton, Ferring Co., Malmö, Sweden) in 500 to 1,000 ml. of physiologic sodium chloride solution or 5.5 per cent glucose solution was administered by continuous intravenous infusion during eight hours. Urine was collected on that day and on the following day. When the result of the ACTH test was evaluated, the "stimulated" level of 17-OH-CS (*i.e.*, the output on the day ACTH was administered) was plotted against the mean resting level on a logarithmic scale. The "normal area" of ACTH test results, based on data in 52 apparently healthy persons (1), is indicated on each graph.

Reproducibility of results with the ACTH test

Many of the patients underwent more than one ACTH test. As a rule, however, a fairly long time elapsed between the tests, or treatment of various types was given in the intervals; such cases were deemed unsuitable for studying reproducibility. Nevertheless there were 10 patients in whom the ACTH test was repeated after about four weeks in which no important therapy had been given (Fig. 1).

When the ACTH test was repeated after a short interval—twice in 2 patients—the results were similar in each case. The group is too small for definite conclusions, but it suggests that the test response is readily reproducible.

RESULTS

I. *Hyperfunction of the adrenal cortex*

The results of the ACTH tests in the 8 patients with hyperplasia of the adrenal cortex (6 with classic Cushing's syndrome and 2 with the adrenogenital syndrome), the 4 patients with adrenocortical adenoma, and 1 with adrenal cancer are shown in Figure 2.

In all but 1 of the patients with hyperplasia, the resting levels of urinary 17-OH-CS were above the limits of the normal range. Six of the 8 in this group, moreover, showed an exaggerated response to exogenous ACTH. This tallies with observations by earlier writers (11) and seems to indicate the presence of an increased functioning mass of adrenal cortex. Three of the 4 patients with benign adenoma had elevated resting levels of urinary 17-OH-CS, but responded only weakly or not at all to stimulation with ACTH. The patient with adrenal cancer had pathologically low levels of urinary 17-OH-CS both at rest and after stimulation with ACTH. Presumably the adrenal cancer was incapable of producing C-21 steroids; the daily excretion of 17-KS was approximately 100 mg., more than 70 per cent of which was identifiable as dehydroepiandrosterone. As expected, the excretion of 17-KS was not influenced by stimulation with ACTH.

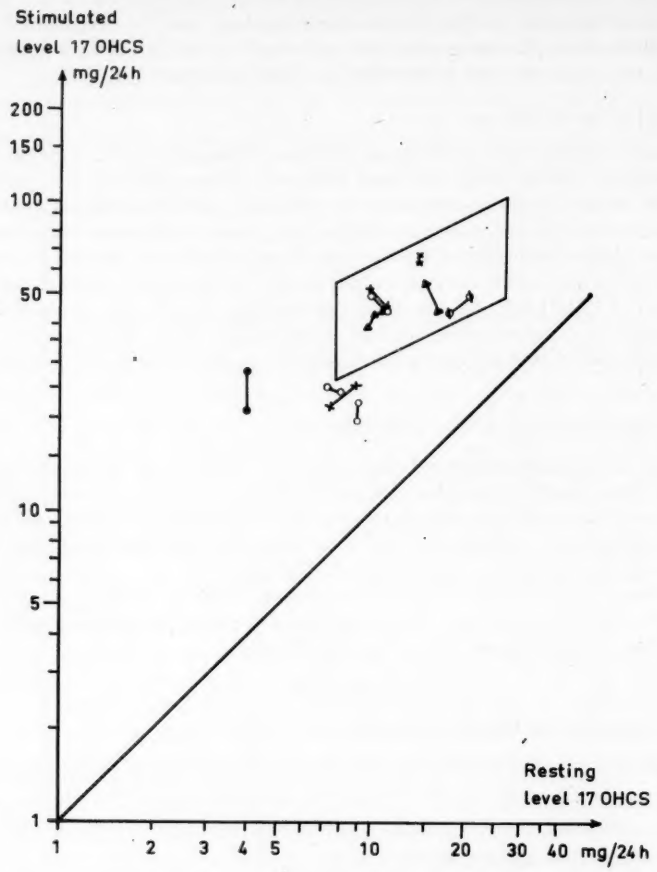

FIG. 1. Reproducibility of ACTH test. Identical symbols connected with a line represent results of repeated ACTH tests in the same patient.

In general, the estimation of urinary 17-KS or separate estimation of individual 17-KS had no major advantage for diagnosis of the cases in this group. Two patients with adrenal hyperplasia showed a "normal" resting excretion of 17-KS and a "normal" response to ACTH, whereas in 2 others with the adrenogenital syndrome the resting 17-KS excretion was greatly elevated although there was slight or no response to ACTH. Furthermore, all 4 patients with benign adenoma had "normal" 17-KS values both before and after stimulation with ACTH. The usefulness of estimating the urinary 17-ketosteroids or their pattern in our cases of adrenocortical hyperfunction with Cushing's syndrome was found to be limited in contrast to its value in the adrenogenital syndrome.

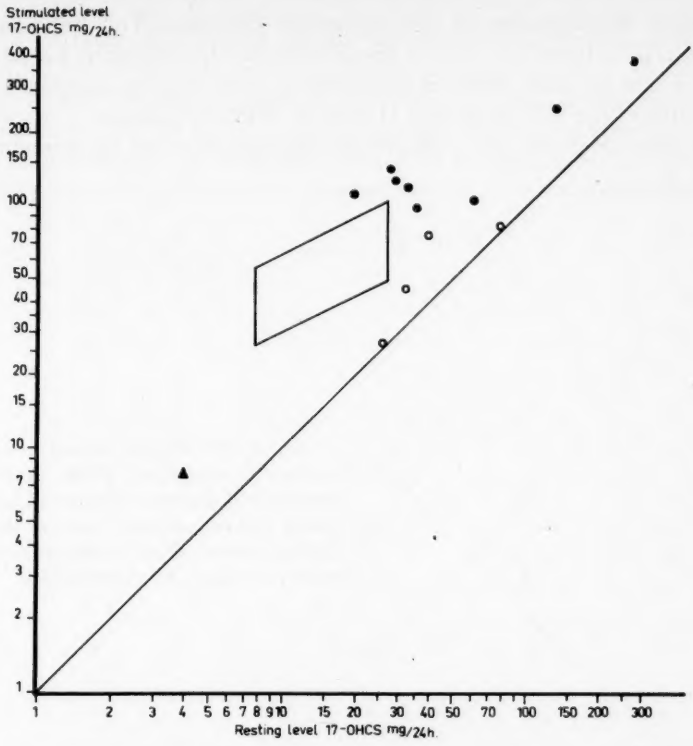

FIG. 2. ACTH tests in cases of hyperfunction of the adrenal cortex. Black dots represent cases of hyperplasia of the adrenal cortex. Circles represent cases of benign adenoma of the adrenal cortex. The black triangle represents 1 case of adrenocortical cancer.

A comprehensive study of these methods in a larger group of cases would appear to be essential for definite assessment of their value in hyperfunctional conditions. This view was strengthened by the observation of a "normal" resting excretion of 17-OH-CS in 10 women with hirsutism, but a pathologically elevated excretion of 17-KS in 4 of the same patients. In none of the hirsute women with normal resting excretion was there an abnormal response of the 17-OH-CS to intravenous ACTH. Similar results have been reported by Gemzell *et al.* (12).

II. *Clinically established or suspected hypofunction of the adrenal cortex*

In the 87 patients with established or suspected adrenocortical hypofunction, estimation of the total urinary 17-KS and of individual 17-KS before and after stimulation with ACTH provided no information of significant diagnostic value. Such evaluation appears to be of little use for dem-

onstrating derangement of adrenocortical function. The findings concerning 17-KS, therefore, are not discussed under the following headings.

a. *Addison's disease, bilateral adrenalectomy and primary hypopituitarism.* The results of the ACTH tests in 11 cases of Addison's disease, 3 cases of bilateral adrenalectomy and 7 of primary hypopituitarism are presented in Figure 3.

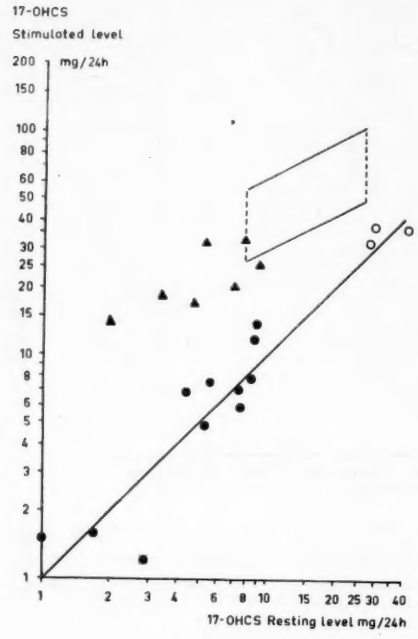

FIG. 3. ACTH tests in cases of adrenocortical hypofunction. Black dots represent cases of Addison's disease. Circles represent cortisone-treated cases of bilateral adrenalectomy. Black triangles represent cases of primary hypopituitarism.

Figure 3 shows that 6 of the 11 patients with Addison's disease had a pathologically low resting excretion of 17-OH-CS. There was almost no rise following administration of ACTH, which indicates virtually complete absence of functioning adrenocortical tissue. The remaining 5 patients with Addison's disease had resting 17-OH-CS excretion levels between 7 mg. and 10 mg. per twenty-four hours. Because of clinical signs of severe adrenocortical insufficiency, 2 of these patients received 20 mg. of cortisol daily throughout the period of the ACTH test. The other 3 patients did not respond to intravenous administration of ACTH by an increased output of corticosteroids, despite a normal resting excretion of 17-OH-CS. This suggests virtual lack of adrenocortical reserve. All 3 patients had pathologic pigmentation.

The 3 patients who had undergone bilateral adrenalectomy showed no alteration of 17-OH-CS excretion following administration of ACTH. The 5 patients with primary hypopituitarism showed a relatively low resting excretion of 17-OH-CS and a normal or nearly normal response to ACTH. Thus it is justifiable to assume that the ACTH test is a useful aid in the differential diagnosis of primary and secondary adrenocortical insufficiency.

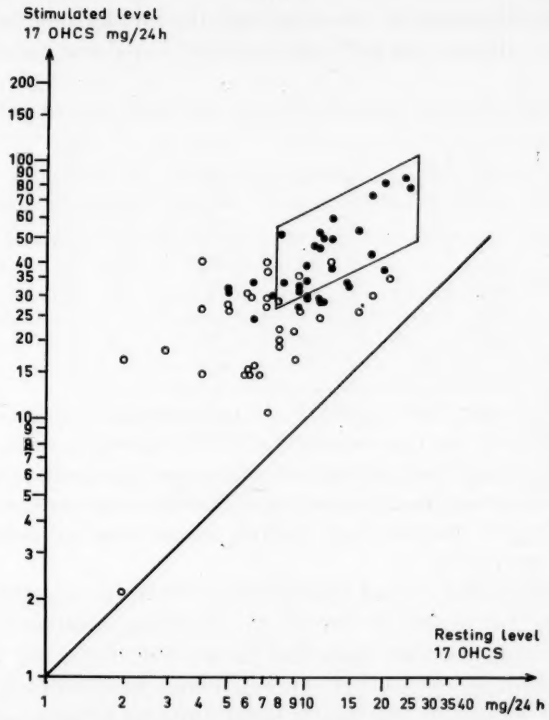

FIG. 4. The results of ACTH tests in 66 patients. Black dots represent tests in patients displaying only one "classic" symptom of Addison's disease. Circles represent tests in patients showing more than one such symptom.

The importance of repeated ACTH tests for the evaluation of these conditions has been pointed out by Jenkins *et al.* (13).

b. *Suspected adrenocortical hypofunction.* This group includes 66 patients who had at least one of the six earlier mentioned classic manifestations of Addison's disease. The distribution of these symptoms was summarized in Table 1. The results of the ACTH test in these 66 patients are shown in Figure 4.

In 23 of the patients there was a completely normal reaction to ACTH, and in 43 the response was subnormal. Six of the latter patients had vasoregulatory asthenia, and in 5 of them the response was subsequently found to be normal following intensive physical treatment.

Of the 43 patients with a pathologic response to the ACTH test, 26 had a pathologically low resting excretion of 17-OH-CS. Following administration of ACTH, the excretion of 17-OH-CS rose to normal in half of the group but was subnormal in the other half. It could be assumed that some at least of these patients had secondary partial hypofunction, with the primary lesion in the pituitary gland. In 17 patients the resting 17-OH-CS excretion was normal, but the response to ACTH was subnormal. In our view most of these patients had diminished adrenocortical reserve capacity.

TABLE 2. DISTRIBUTION OF MAIN SYMPTOMS AND RESPONSES TO ACTH TEST

Response to test	Total cases	Weight loss	Weakness	Pigmentation	Hypotension	Hypoglycemia	Disturbed water balance
Normal	23	2	20	0	4	1	0
Pathologic	43	14	43	6	21	3	2
Total	66	16	63	6	25	4	2

In Table 2 is shown the distribution of clinical symptoms in these 66 patients according to whether they reacted normally or subnormally to administration of ACTH.

In the patients who reacted normally to ACTH, weakness was the most common symptom (Table 2); the other symptoms rarely occurred in this group. In striking contrast were the patients who showed a pathologic reaction to administered ACTH. Although they had complained of weakness, many of them also had loss of weight and/or hypotension. Approximately 10 per cent had some degree of pigmentation, which was typical in color and localization. Manifest disturbances of carbohydrate metabolism or of electrolyte and water balance, on the other hand, were less common.

Figure 5 shows the frequency of symptoms in the individual patients who reacted normally to ACTH, and a comparison of this group with those who reacted pathologically.

Of the 23 patients with a normal response to the ACTH test, only 3 had more than one of the typical symptoms; 1 patient had three. This was the highest frequency of symptoms in the group. Of the 20 patients with a single symptom, 18 complained of weakness and 2 had hypotension. Weakness, as expected, was the least specific of the symptoms. The 3

patients with a normal ACTH response and more than one symptom had weakness plus loss of weight or hypotension; in 1 case there was also a pathologic type of glucose tolerance curve.

Among the 43 patients who showed a pathologic response to the ACTH test, the distribution of symptoms was quite different. Most of the patients had two or more symptoms. A relatively small group had only a single symptom (weakness).

Figure 5 shows that when four symptoms were present the response to the ACTH test was always pathologic. The number of cases with four

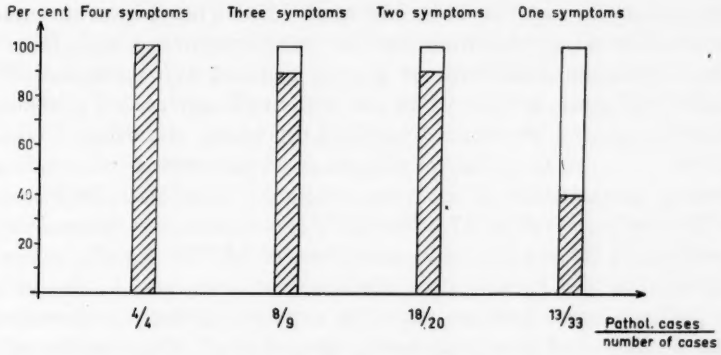

FIG. 5. Frequency of pathologic response to ACTH test in relation to the number of symptoms of Addison's disease. The striped part of the columns is proportional to the number of pathologic responses.

symptoms was very small, however. It may further be questioned whether these cases justifiably belong to their present group, or if they should have been classed among the patients with manifest adrenocortical insufficiency, primary or secondary. Of the patients with two or three symptoms, 90 per cent showed a pathologic response to exogenous ACTH. When there was only one symptom, on the other hand, the incidence of pathologic ACTH reactions fell to 40 per cent.

In Figure 4 the results of the ACTH test in patients with only one of the classic symptoms of adrenocortical hypofunction may be compared with the results in patients who had more than one symptom. It may be seen that the truly pathologic results were found in patients with several symptoms, whereas the values near the normal limits were found in those with a single symptom.

DISCUSSION

The pioneer work of Thorn and his associates (13–16) led to the formulation of a concept of paramount importance, *viz*, the functional reserve

capacity of the adrenal cortex. It is now generally agreed that under normal circumstances the human adrenal cortex is endowed with a generous functional reserve which, by the action of ACTH, can be mobilized in conditions of increased demand. It is also well established that the most useful criterion for assessment of this reserve capacity is the amount of adrenocortical steroids and their metabolites in the urine and plasma following intravenous infusion of pituitary adrenocorticotropic hormone.

In 1955 Jenkins *et al.* (13) attempted to place the assessment of this functional reserve on a quantitative basis by estimating the response of the urinary 17-hydroxycorticosteroids to a standardized intravenous dose of ACTH, using the method of Reddy *et al.* (17). They found pronounced variations in response; the lower limit of normal response was, in fact, close to zero. Although these findings greatly reduced the usefulness of the method for diagnosing diminished but not completely absent adrenocortical reserve capacity, the method enabled this group of workers to demonstrate the existence of partial or compensated adrenocortical insufficiency without an adequate functional reserve capacity. They described 7 cases in which the resting levels of 17-hydroxycorticosteroids were normal but did not respond to intravenous administration of ACTH. Evidence was also presented that the Porter-Silber chromogenic substance in the urine of these patients was identical with the expected urinary corticosteroids. Similar findings had been reported by Brown *et al.* (18), who described 2 patients with Addisonian symptoms and with normal levels of 17-hydroxycorticosteroids in the urine and plasma. Administration of ACTH to these patients did not alter the circulating 17-hydroxycorticoid levels. The existence of Addison's disease in a form which is unresponsive to stimulation with ACTH but in which there is a normal plasma content of 17-hydroxycorticoids has been confirmed by several groups of investigators (19–22). The generally accepted inference is that in such patients the secretory function of the adrenal cortex is sufficient to maintain homeostasis under resting conditions, but insufficiency becomes manifest under conditions of an increased demand for adrenocortical steroids.

In an earlier paper it was shown that by using a different method of assay for urinary 17-hydroxycorticosteroids—a modification of the technique of Appleby *et al.* (1, 7)—the readings during rest and following ACTH stimulation are much higher than those found with the Reddy method (17).

It was further demonstrated that a highly significant correlation exists between the logarithms of urinary 17-hydroxycorticosteroid excretion at rest and after ACTH stimulation. From these observations a fiducial area of "normal response to ACTH" was calculated, within which the results of 95 per cent of future ACTH tests in healthy subjects could be expected to

lie. There was a considerable interval between the lower fiducial limit of the "normal area" and the "no response" line (*i.e.*, a line connecting the points where the stimulated levels are equal to the resting levels). It was therefore hoped that this scheme might provide a suitable method for laboratory evaluation of different degrees of diminished adrenocortical reserve capacity.

The data in the present paper confirm in the first place the observations of previous investigators (16, 18) concerning compensated adrenal insufficiency. Three of our patients had an apparently normal resting excretion of urinary 17-hydroxycorticosteroids but did not respond to ACTH with increased excretion. The findings of earlier writers (16, 18–22) and our observations thus suggest strongly that a normal basal excretion or plasma level of 17-hydroxycorticosteroids is not *per se* convincing evidence of normal adrenocortical function. Therefore, whenever clinical symptoms of adrenocortical insufficiency are present, it would seem to be advisable, if not mandatory, to check the reserve capacity of the adrenals.

Our patients with manifest adrenocortical hypofunction or hyperfunction responded as expected to ACTH tests, *viz*, with values below the normal range in hypofunction and above this range in hyperfunction. These results constitute strong evidence of the clinical sense of the ACTH test.

This modified test also permits study of possible partial insufficiency of the adrenal cortex or the pituitary gland. Many clinical observations, including those collected by Kappert (23), have shown the occurrence of this condition to be highly probable, but there has been no definite proof of its existence. Reduction of adrenocortical function in asthenic states has earlier been postulated from studies on the reserve capacity of the adrenal cortex (24).

In the present study, 43 of 66 patients with clinically suspected adrenocortical hypofunction were found to have reduced reserve capacity according to the test used. These patients had at least one of six classic symptoms of hypofunction of the adrenal cortex, but no definite clinical manifestations of other organic or psychic disorders. Detailed clinical examination showed that weakness was the most common symptom, followed by hypotension and loss of weight. About 10 per cent of the patients had typical pigmentation, but disturbances of carbohydrate metabolism or of water balance were rarely observed.

Most of the patients with reduced reserve capacity according to the ACTH test had several of the typical symptoms, whereas the patients whose response to the test was normal as a rule had only one symptom, usually weakness. As expected, weakness was the least specific symptom in this connection. In the relatively few patients with only one symptom but with a pathologic response to the ACTH test, the values as a rule were not

far from the normal limit. When two or more of the symptoms were present together, the probability was 90 per cent that the patient had reduced adrenocortical reserve capacity. When at least four of the six symptoms coexisted, this capacity was invariably reduced.

In about half of the patients with reduced adrenocortical reserve capacity the resting urinary excretion of 17-OH-CS was pathologically low. In many of the same patients, however, maximal stimulation with ACTH raised the output of corticosteroids to relatively normal levels, indicating that defective hypophysial ACTH production probably was the underlying cause of the patients' symptoms. In the other half of the group, the resting excretion of 17-OH-CS was normal, but the response to stimulation with ACTH was subnormal, which suggested that in many of these patients there was partial diminution of adrenocortical function.

In the patients with suspected reduced adrenocortical function, primary or secondary, there was a highly satisfactory correlation between the symptoms and the results of the ACTH tests. The patients who had only one symptom suggestive of hypofunction responded normally or almost normally to the ACTH test, whereas most of the patients with multiple symptoms showed a pathologic response to the test. A single clinical deviation from normal, such as weight loss or weakness, often is a fairly nonspecific symptom, whereas certain symptoms (pigmentation and, to a lesser extent, hypotension) are more specific. The clinical picture of a disease is formed by summation of specific and nonspecific symptoms. This was well confirmed in the present analysis, in which pigmentation, hypotension, disturbed carbohydrate metabolism and fluid imbalance were almost always associated with adrenocortical hypofunction. Even coexistence of several nonspecific symptoms may have similar implications in clinical material selected so as to exclude debilitating diseases. The clinician should therefore suspect partially reduced adrenocortical or pituitary function when several of the foregoing symptoms are present together and are unaccompanied by signs of chronic debilitating disease. Whether the reduced adrenocortical reserve capacity is a progressive or a stationary condition, or is secondary to other pathologic changes, must be determined at a later stage of the investigation.

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THE OCCURRENCE AND TREATMENT OF HYPOTHYROIDISM AMONG ALCOHOLICS

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ABSTRACT

In 33 alcoholic patients, thyroid function was estimated clinically and the results compared with laboratory findings using standard thyroid function tests and also the response of the serum PBI level to administration of TSH. Twenty-one (64 per cent) of patients were found to be thyroid-deficient in varying degree. When treated with desiccated thyroid (180 mg. daily on the average) or *l*-triiodothyronine (100 μg. daily, average), impressive results were obtained both in regard to improvement of hypometabolic symptoms and drinking patterns. Fifteen patients (46 per cent) had an "excellent" response, eight (24 per cent), a "good" response; and ten (30 per cent), a "fair" or "poor" response. As judged by curtailment of drinking, hypothyroid patients responded better than did euthyroid patients; sudden discontinuation of medication exacerbated the symptoms of alcoholism in several patients. An interrelationship between alcoholism and thyroidal hypofunction is postulated and discussed; several cause-and-effect mechanisms are explored. It is concluded that the presence of thyroid hormone deficiency is an important (but not the only) factor for consideration in the treatment of alcoholism, and that correction of the hypothyroid state with proper hormonal therapy appears to be a logical way to prepare the patient, both physically and mentally, for achievement of the maximal benefit from psychotherapy.

A CLOSE relationship between neuro-endocrine dysfunction and alcoholic addiction in man has long been suspected, though as yet no consistent hormonal deficiency has been fully documented (1–4). Several investigators have proposed a state of relative adrenal insufficiency or of pituitary unresponsiveness to stress as possible mechanisms, but these studies have been contested because of the techniques that were employed. In 1956, a report by Richter (5) focused attention on the possibility that thyroid deficiency might play an important role either in the basic etiology or in the dynamic course of addiction to alcohol. Using a well executed series of laboratory experiments in rats preconditioned to consume large amounts of alcoholic solutions with their daily feedings, Richter demonstrated a significant decrease in the intake of alcohol when thyroid powder

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or thyroid derivatives were introduced into the diet; the opposite effect—a marked preference for the alcoholic solution over the stock diets—was demonstrated when the rats were made hypothyroid following thyroidectomy.

Through a coincidental observation, our interest in the role of a thyroid factor in the pathogenesis of chronic alcoholism was first aroused while investigating the frequency of occurrence and diverse symptom complex of early hypothyroidism. Among the first several patients who demonstrated both clinical and laboratory evidence of thyroid deficiency, there were 2 who admitted a rapidly progressing inability to limit their alcohol intake. Within a few months following the correction of hypothyroidism by replacement therapy, both of these patients remarked extemporaneously that not only were their previous symptoms improved but they experienced a new-found ability to control their drinking. This surprising response, when put into perspective with the somewhat similar observations of Richter in animals, prompted the investigation of the thyroid status of a group of "borderline" and frank alcoholics available in the author's outpatient clinic.

MATERIALS

Among military personnel and dependents in the Paris area, 33 alcoholics (30 men and 3 women) ranging in age from 26 to 48 years were studied from January 1958 through September 1959. All but 5 were followed for a minimum of six months. The reasons for the shorter course of study in these 5 patients are given in Table 2.

The majority of the patients were either admitted alcoholics or had been referred for treatment by their superior officers following one or more episodes of alcoholic intoxication. Several had been previously court-martialed or threatened with court-martial because of suspected or verified drinking on duty. At least half of the group had run the gamut of current therapy (Antabuse, tranquilizers and psychotherapy) without noticeable improvement.

Although it was realized that an ideal study concerning the possible interplay of thyroid function and alcoholism should be conducted by the "double-blind" method of administering medication, this technique was decided against because: a) many of these patients were acutely in need of treatment since their military careers were in jeopardy, b) thyroid therapy was indicated to control hypothyroid or hypometabolic symptoms which were unrelated to alcoholism, and c) the study could not have been kept "blind," for the employment of the electrometric recording of the Achilles reflex in conjunction with administration of the rapidly acting thyroid hormone, *l*-triiodothyronine[1] (LT_3), proved to be a simple and accurate means of determining whether the patient was receiving active medication.

METHODS AND INTERPRETATION OF LABORATORY FINDINGS

To evaluate thyroid function as completely as possible, a detailed history was taken and a complete physical examination made on each patient. Then, in order to emphasize

[1] The *l*-triiodothyronine employed in this study as Cytomel was generously furnished in large part by Smith Kline & French Laboratories.

the vagaries of estimating thyroid status on the basis of clinical impressions alone, a tentative clinical diagnosis was made. These diagnoses are shown in Table 1. The laboratory tests included: a) serum protein-bound iodine (PBI) concentration, both in random samples of blood and in samples taken before and twenty-four hours after the injection of 10 international units of thyroid-stimulating hormone (TSH); b) basal metabolic rate (BMR), with oral pentobarbital premedication; c) serum cholesterol concentration; and d) electrometric recording of the free Achilles tendon reflex time as measured on the kinemometer, a simple apparatus devised and reported on by Lawson (6), and employed in our clinic since shortly after the appearance of his paper. The thyroidal uptake of radioactive iodine was not measured except in special instances in order to clarify a particular problem.

The serum PBI values which are accepted as normal in this laboratory range from 4.0 to 8.0 micrograms (μg.) per 100 ml. Although a value somewhat less than 4.0 μg. (usually 3.5 μg.) is commonly accepted as the lower limit in euthyroidism, experience with the determination of PBI has led us us to employ the higher value. Contamination by iodine (*e.g.*, prior administration of x-ray contrast media, of medications such as vitamins or cough syrups containing iodine or iodine-containing pills used to decontaminate drinking water) may cause errors. However, although an artifactually high PBI level in a hypothyroid patient is not uncommon, the converse—a falsely low result in an otherwise normal patient—is quite rare. Among several hundred PBI determinations performed in our outpatient clinic over the past two years, few have been below 4.0 μg. per 100 ml. in patients who have otherwise lacked either clinical or laboratory evidence of hypothyroidism and/or who have not made the expected symptomatic response to thyroid therapy.

In view of this difficulty with iodine contamination, and because we agree essentially with the opinion of Starr (7) and others that the level of circulating PBI is an accurate reflection of the output of thyroid hormone (when the determination is properly performed), we tried to circumvent the hidden sources of contamination by utilizing the relative increase in PBI concentration following injection of TSH rather than accepting the results of single assays. As documented by Jefferies and associates (8), this procedure is not only valuable in the foregoing regard, but also in the diagnosis of what is considered to be "low-thyroid reserve" (9). However, the latter diagnosis is uncommon in patients without a prior history of thyroid surgery or radioiodine therapy. Therefore, we believe that among the several patients listed in Table 1 whose initial PBI levels were in the normal range of 4.0 to 8.0 μg. per 100 ml. but failed to rise more than 1.0 μg. following administration of TSH, most of the results represent instances of iodine contamination rather than true "low thyroid reserve." The TSH employed in this study[2] was one of standard potency and induced the expected results when tested in a series of 10 euthyroid controls.

[2] Thyreostimuline, kindly supplied by the Endopancrine Laboratories, Paris, France.

TABLE 1. THYROID FUNCTION TESTS IN 33 ALCOHOLIC PATIENTS

Patient			Initial impression*	Serum PBI level (μg./100 ml.)		Achilles reflex time; S-D interval† (milliseconds)	Final diagnosis
No.	Name	Sex		before TSH	after TSH**		
1	S.A.	M	Obese; euthyroid	3.6	3.8	260	Hypothyroid
2	A.S.	F	Hypothyroid	3.0	3.3	270	Hypothyroid
3	W.Mc.	M	Euthyroid	5.8	8.0	160	Euthyroid
4	L.C.	M	Obese; euthyroid	3.4	3.5	—	Hypothyroid
5	D.J.	M	Euthyroid	4.4	8.8	260	"Euthyroid hypometabolism"
6	C.F.	M	Obese; hypothyroid	5.9	5.7	250	Hypothyroid
7	S.W.	M	Euthyroid	3.3	3.5	—	Hypothyroid
8	D.M.	M	Euthyroid	4.8	5.2	—	Hypothyroid
9	D.C.	M	Obese; hypothyroid	3.6	4.0	270	Hypothyroid
10	A.D.	M	Obese; euthyroid	6.3	10.0	220	Euthyroid
11	H.G.	M	Euthyroid	4.8	6.8	250	"Euthyroid hypometabolism"
12	J.C.	M	Obese; hypothyroid	4.0	4.0	—	Hypothyroid
13	H.R.	M	Obese; euthyroid	6.1	6.3	260	Hypothyroid
14	J.J.	M	Euthyroid	6.0	8.2	200	Euthyroid
15	E.P.	M	Obese; hypothyroid	4.0	4.3	240	Hypothyroid
16	J.G.	M	Euthyroid	4.3	5.0	280	Hypothyroid
17	W.K.	M	Euthyroid	5.8	8.5	190	Euthyroid
18	F.R.	M	Euthyroid	6.2	8.8	220	Euthyroid
19	J.G.	M	Euthyroid	5.0	4.7	250	Hypothyroid
20	T.J.	M	Euthyroid	6.2	8.0	200	Euthyroid
21	A.C.	F	Hypothyroid	6.2	6.0	250	Hypothyroid
22	S.P.	M	Obese; hypothyroid	4.0	6.8	260	"Euthyroid hypometabolism"
23	G.R.	M	Obese; euthyroid	4.2	6.0	230	Euthyroid
24	E.Z.	M	Hypothyroid	3.3	3.3	—	Hypothyroid
25	K.C.	M	Hypothyroid	3.0	3.2	280	Hypothyroid
26	M.G.	F	Hypothyroid	3.5	3.2	280	Hypothyroid
27	R.S.	M	Euthyroid	5.5	9.0	180	Euthyroid
28	T.P.	M	Hypothyroid	3.0	3.2	—	Hypothyroid
29	R.L.	M	Hypothyroid	2.8	2.6	270	Hypothyroid
30	W.C.	M	Euthyroid	6.7	10.5	230	Euthyroid
31	P.W.	M	Hypothyroid	2.5	2.8	280	Hypothyroid
32	S.P.	M	Euthyroid	5.1	4.8	260	Hypothyroid
33	L.B.	M	Hypothyroid	2.3	2.8	300	Hypothyroid

* Clinical impression following initial interview and physical examination only.

** Normal rise in PBI level after TSH is more than 1.0–1.5 μg. per 100 ml.

† Normal range, 140–240 milliseconds.

The electrometric recording of the delayed tendon response in a group of myxedematous patients was first reported by Lambert *et al.* in 1951 (10). The simplicity of the Lawson kinemometer (a modification of the apparatus used by Lambert), as well as its easy availability and the economy of its few parts, makes it a utilitarian apparatus for use in the office or outpatient clinic. The normal limits for the contraction phase, or S-D interval of the reflex, range from 140 to 240 milliseconds; values above 240 milliseconds suggest hypothyroidism. When Lawson compared the results of the S-D measurement with the thyroid status of over 3,000 patients, the values obtained on the kinemometer proved 94 per cent accurate in the diagnosis of hypothyroidism (11). Our own experience in comparing the Achilles reflex recording with the results of PBI-TSH tests and BMR's in 140 patients seems to confirm the high degree of accuracy—well over 90 per cent—claimed for the kinemometer in distinguishing between euthyroidism and even the milder degrees of hypothyroidism. By employing the kinemometer in association with administration of the rapidly metabolized thyroid hormone, *l*-triiodothyronine (LT_3), it was possible to follow changes in the reflex time and to determine whether a patient is adhering to the treatment regimen prescribed for him. The following case will serve to illustrate this point:

K. C., a 32-year-old army sergeant, was first seen in November 1958 because of alcoholism of approximately five years' duration. At that time, the contraction phase (S-D interval) of his Achilles tendon reflex measured 280 milliseconds. An initial serum PBI level was reported as 3.9 μg. per 100 ml.; twenty-four hours after administration of TSH it was 3.2 μg. per 100 ml., which confirmed the diagnosis of hypothyroidism. LT_3 was started in a dosage of 50 μg. daily, which was gradually increased so that by mid-February he was taking 100 μg. per day. At that time his reflex time had decreased to 200 milliseconds. In early March, while taking the same amount of LT_3, the S-D interval was still 200 milliseconds.

In early April, he called for an appointment, since he felt he was on the verge of a drinking spree. When seen that afternoon, there was a strong odor of alcohol on his breath and he appeared apprehensive and tremulous, as though he were bordering on delirium tremens. Though questioned explicitly on this point, he denied stopping his thyroid medication for a single day. When confronted with the evidence that his "metabolism"—as demonstrated by a reflex time of 260 milliseconds on the kinemometer—did not corroborate his statement, he admitted that he had run out of his pills ten days previously and had not returned to the pharmacy for a refill.

It is also of interest that forty to fifty minutes after administering 100 μg. of LT_3 by the sublingual route, his tremulousness and anxiety had abated considerably and up to the time of this writing, he has done well and is continuing therapy.

As shown in Table 1, among this small series of 33 alcoholic patients, 21 (64 per cent) were found to be thyroid deficient. Assessment of other parameters of thyroid function such as the BMR and serum cholesterol level, which are generally believed to measure the peripheral effects of thyroid

hormone rather than the actual output of hormone, tended to confirm the diagnosis of thyroid insufficiency in the majority of patients. However, the results were not as consistent in this regard as were the results of the PBI-TSH test and the kinemometer recording. This finding held true not only for the alcoholics who were hypothyroid but also for a much larger series of hypothyroid patients presently being followed in our clinic. Furthermore, high serum cholesterol values in a group of rats fed alcohol have been reported previously by Gottlieb *et al.* (12), although whether the elevation was due to thyroid deficiency or to liver disease was not determined since thyroid function tests were not included.

Twenty-six of our 33 patients were treated with LT_3 only; initial daily doses ranged from 50 to 75 μg. The final average daily maintenance dose was 100 μg. Four patients were treated with desiccated thyroid only, in dosages of 120–180 mg daily. The remaining 3 patients received desiccated thyroid first for six to eighteen weeks; then the medication was changed to LT_3 when it became more readily available.

The response to therapy was considered "excellent" when the patient (as reported by either his Commanding Officer or his spouse) had controlled his alcoholic intake consistently over a minimum post-treatment period of six months; "good" when the patient, although consuming considerably less alcohol than previously and staying out of any trouble secondary to alcoholism, was still imbibing to the point of intoxication on certain occasions; "fair" when the patient seemed better able to limit his daily alcoholic intake while taking adequate amounts of LT_3, yet took his pills sporadically; and "poor" when the patient failed to benefit from therapy and faced some form of disciplinary action as a consequence of his continued drinking. The results are summarized in Table 2. It may be seen that 15 patients (46 per cent) had an "excellent" response to thyroid hormone replacement therapy; 8 (24 per cent) had a "good" response; and 10 (30 per cent) had a "fair" or "poor" response.

Table 3 shows the pretreatment thyroid status in relation to the patients post-treatment ability to control the intake of alcohol. The over-all response to thyroid therapy was considerably better in hypothyroid patients than in those who were probably euthyroid, although in any individual case this was not invariably so. Four of 9 patients (44 per cent) in the euthyroid group had a good to excellent response to *l*-triiodothyronine in an average dosage of 100 μg. per day. Between these two groups there were 3 patients who might be classified in the recently proposed category of "euthyroid hypometabolism." These patients had a normal level of serum PBI and a normal response to TSH, and yet had an abnormally low BMR and a prolonged reflex time. Whether such a syndrome exists and, if so, whether LT_3 is the proper therapy is debatable and beyond the scope of this report.

TABLE 2. CHANGE IN ALCOHOLIC INTAKE IN PATIENTS TREATED WITH THYROID OR *l*-TRIIODOTHYRONINE

Patient		Diagnosis	Therapy	Duration of treatment (mos.)	Response*	Comment
No.	Name					
1	S.A.	Hypothyroid	Thyroid	12	E	
2	A.S.	Hypothyroid	Thyroid; LT_3	14	E	Felt better with LT_3
3	W.Mc.	Euthyroid	LT_3	2	P	Stopped Rx 8 wks. after onset despite good response; attempted suicide 10 days later
4	L.C.	Hypothyroid	Thyroid	9	E	Severe chronic insomnia relieved by LT_3
5	D.J.	"Euthyroid hypometabolism"	LT_3	6	P	Took Rx sporadically
6	C.F.	Hypothyroid	LT_3	7	E	40-lb. weight losss during Rx
7	S.W.	Hypothyroid	LT_3	11	G	
8	D.M.	Hypothyroid	Thyroid; LT_3	10	G	LT_3 helped more than equivalent amount of thyroid
9	D.C.	Hypothyroid	LT_3	11	E	25–30 lb. weight loss during Rx
10	A.D.	Euthyroid	LT_3	7	P	Stopped, then started Rx on 3 occasions
11	H.G.	'Euthyroid hypometabolism"	LT_3	8	G	Excellent symptomatic response; moderately decreased intake alcohol
12	J.C.	Hypothyroid	Thyroid; LT_3	9	E	20-lb. weight loss and marked improvement in mental attitude
13	H.R.	Hypothyroid	LT_3	6	E	48-lb. weight loss
14	J.J.	Euthyroid	LT_3	2	P	Stopped Rx; 12 days later court-martialed for alcoholism
15	E.P.	Hypothyroid	Thyroid	10	E	
16	J.C.	Hypothyroid	LT_3	6	G	
17	W.K.	Euthyroid	LT_3	3	P	Court-martialed for alcoholism; infrequent follow-up; may have stopped Rx (?)
18	F.R.	Euthyroid	LT_3	9	G	
19	J.G.	Hypothyroid	LT_3	7	E	Excellent response re relief of tension and flare-ups of ulcer
20	T.J.	Euthyroid	LT_3	9	F	Infrequent follow-up but kept out of trouble
21	A.C.	Hypothyroid	LT_3	7	E	Impressive response re fatigue and nervousness, etc.
22	S.P.	"Euthyroid hypometabolism"	LT_3	6	E	Impotence and decreased libido improved considerably by Rx
23	G.R.	Euthyroid	LT_3	6	G	10-lb. weight loss during Rx
24	E.Z.	Hypothyroid	Thyroid	10	G	
25	K.C.	Hypothyroid	LT_3	6	E	See case history in text, under Methods
26	M.G.	Hypothyroid	LT_3	6	G	Increase in alcoholism after Rx temporarily stopped by psychiatrist
27	R.S.	Euthyroid	LT_3	7	E	
28	T.P.	Hypothyroid	LT_3	10	E	Alcoholic for 8 yrs.
29	R.L.	Hypothyroid	LT_3	6	F	Stopped, then started Rx on 2 occasions
30	W.C.	Euthyroid	LT_3	2	P	Reported for alcoholism 6–7 days after stopping Rx; lost to follow-up
31	P.W.	Hypothyroid	LT_3	6	E	Marked relief of back pain during Rx; wife also hypothyroid
32	S.P.	Hypothyroid	LT_3	6	P	Court-martialed for alcoholism; admitted stopping Rx 10 days prior to spree
33	L.B.	Hypothyroid	LT_3	1½	P	Reported for drinking and being involved in car accident

* E = excellent; G = good; F = fair; P = poor.

Of utmost interest, however, is the fact that among 7 failures in this series, kinemometer tracings, as well as the patients' own admissions, indicated that 4 or 5 of these men had discontinued medication at least one week prior to their bout with alcohol. Rawson, Koch and Flach (13), reporting on the use of *l*-triiodothyronine in 4 carefully studied psychoneurotic patients, 2 of whom were alcoholics, also observed an exacerbation of symptoms and neurotic behavior following discontinuance of medication. It is, therefore, of critical importance for the therapist to inform his patients of this fact, and to keep this in mind as a possible danger inherent in this form of management.

When alcoholic patients are thyroid-deficient, proper replacement therapy with thyroid hormones should help them not only to curtail their daily

TABLE 3. CORRELATION OF THYROID STATUS AND RESPONSE TO THERAPY WITH THYROID HORMONES

Diagnosis	Therapeutic results				
	Excellent	Good	Fair	Poor	Totals
Hypothyroid	12	5	1	3	21 (64%)
"Euthyroid hypometabolism"	1	1	0	1	3 (9%)
Euthyroid	2	2	1	4	9 (27%)
Totals	15 (46.5%)	8 (24.3%)	2 (6%)	8 (24.2%)	

drinking but also to relieve the numerous other symptoms which usually accompany the hypothyroid state, such as fatigue, irritability, depression, obesity, intolerance of cold and decreased libido. This, indeed, is the case. As a consequence of the general improvement in his health, the patient is encouraged to continue his treatment and to return periodically to the clinic. Mental attitude, in particular, is likely to improve, aiding the patient to make a better adjustment both in his job and in his family relationships. Especially in the truly hypothyroid patient, thyroid therapy may bring about a sense of relaxation and relief of inner tension which is in many ways comparable, but preferable, to the artificial "haze" created by alcohol.

DISCUSSION

Conceding that a rather intimate relationship between progressive alcoholism and thyroid failure does exist, the question arises as to the frequency of their combined occurrence among alcoholics at large and, more important, which of the two conditions appeared first. Does hypothyroidism

lead to alcoholism in the susceptible person, who is perhaps pre-conditioned by a family of heavy drinkers or a neurotic milieu; or does alcohol, when taken in excess for long periods of time, exert a damaging or "toxic" effect on either the thyroid gland or the pituitary-thyroid axis?

In answer to the first part of the foregoing query, the incidence of hypothyroidism diagnosed in the group of alcoholics seen by us has remained relatively constant as the total number of patients undergoing investigation has increased. Routine use of thyrotropic hormone in establishing or dismissing the diagnosis is strongly recommended, especially in patients who present evidence of fatty metamorphosis or early hepatic cirrhosis. Previous studies have convincingly delineated the key importance of the liver in thyroid hormone metabolism (14), and have also shown the frequency of abnormal results with the commonly employed thyroid tests following the occurrence of either acute or chronic hepatic damage.

Certainly, not *all* alcoholics are thyroid-deficient. It should be emphasized that the term "alcoholic" as used here in the medical sense of the word, does not include the intemperate social drinker, who might be better considered as in the reversible stage of alcoholism. Assuming that both psychodynamic and metabolic influences contribute in varying degrees to a particular person's dependence upon alcohol, both facets seem worthy of consideration. One might draw a comparison here with the separate roles played by psychoneurotic and endocrinologic factors in the common symptoms of nervousness. Premenstrual tension is, perhaps, a pertinent example of such a dual mechanism producing a symptom complex requiring consideration of both psyche and soma in its successful treatment. By analogy, the borderline alcoholic who responds to psychotherapy or to a threat of peptic ulcer, by sharply curtailing his future drinking, may have had a predominantly psychodynamic drive to imbibe excessively. In contrast, persons who cannot taper off the intake of alcohol despite alcoholic cirrhosis or gastro-intestinal hemorrhage or personal disaster, may have a definite metabolic disturbance which reinforces a pre-existing personality disturbance.

As proposed by Richter (15), excessive consumption of alcohol may well represent a symptom to be added to the already protean manifestations of the hypothyroid state. The inner tension and frustrations so common in thyroid insufficiency (16) may precipitate alcoholic tendencies in the person with basically neurotic behavior patterns. Table 4 summarizes data obtained by questionnaires on the drinking habits of 104 nonalcoholic hypothyroid patients presently being followed by this author. Although the great majority of the patients in this sampling were not heavy drinkers, a considerable number (35 per cent) of those who were, stated that they had noticed a decrease in the desire for alcohol after starting thyroid therapy.

Alcoholics who responded successfully to thyroid therapy were later questioned as to what, in their own words, they felt had helped them control their drinking. The two factors most frequently mentioned were: (a) alleviation of nervousness and irritability, usually associated with marked evening-fatigue, which often precipitated family quarrels unless relieved by several drinks on the way home, and (b) alleviation of the necessity to consume several drinks before the effect of the alcohol was fully reached. They remarked that after only two or three drinks they now felt a "glow" and could stop drinking for the evening. The latter statement is of considerable interest in the light of observations made by Koch that *l*-triiodothyronine hastens the body's metabolism of alcohol. The report by Rawson, Koch and Flach (13) on the prompt, sobering

TABLE 4

A. *Daily alcoholic intake in 104 nonalcoholic hypothyroid patients before thyroid replacement therapy*

1. Above average	28%
2. Average	57%
3. Below average	15%

B. *Change in alcoholic intake following the onset of thyroid replacement therapy*

1. Significant decrease	38%
2. Moderate change	16%
3. No change	52%
4. Increased intake	4%

effect of LT_3 in 2 alcoholics with impending delirium tremens, further corroborates such a change and is in keeping with experiences of the author in the case of Patient K. C. and several other patients. The alcohol content of blood drawn before, and one and two hours after administration of 100 to 200 μg. of LT_3 by the sublingual or intravenous route, showed a significant decrease in 6 of 8 acute alcoholics so treated; moreover, the fall in the blood alcohol level occurred two to three times faster than in untreated, matched controls.

Thus, the insult of hypothyroidism to the body metabolism, especially in "borderline" alcoholics, seems able to precipitate true alcoholic addiction. But what of the converse approach—that the alcohol itself is a thyroid "poison" leading to the well known and well named vicious cycle? Certain evidence in recent medical and neuropsychiatric literature lends support to such a theory. Goldfarb and Berman (2), in discussing the glandular pathologic conditions of animals and men which follow chronic excessive exposure to alcohol, offered the hypothesis that alcohol serves

initially to stimulate the pituitary-endocrine axis (the adrenal gland in particular) but that later, through the mechanism of "exhaustion-depletion," multiglandular degeneration ensues with consequent pan-endocrine hypofunction. Gross (1), in reporting on a possible relationship between pituitary function and certain symptoms of alcoholism, discusses evidence suggesting that alcohol temporarily inhibits the production or end-organ effectiveness of TSH.

Hion-Jon (3), in summarizing the histologic alterations observed in rabbit endocrine glands following chronic, fatal alcoholic poisoning, describes the following changes in the thyroid: "Cellular degeneration, formation of holes, swelling, degeneration of nuclei in almost all cases. Enlargement of the follicles and flattening out of the epithelium. . . . Bubbles in the interstitial tissues filled with colloid pushed out by the follicles. . . ." The statement regarding the extrusion of colloid from the damaged follicle may be of particular significance in the light of the impressive number of convincing, recent reports indicating the presence of an auto-immune mechanism at work in many cases of thyroiditis and spontaneous myxedema (17, 18). It is entirely possible that repeated bouts of alcoholic intoxication may eventually damage the thyroid follicles, thereby releasing colloid into the circulation which, in turn, may instigate an auto-immune reaction to thyroglobulin substances. Such a mechanism, once in progress, would then (as in Hashimoto's thyroiditis) cause a lymphocytic invasion of the gland and a progressive thyroid deficiency. It might prove enlightening to employ the various complement-fixation and precipitin techniques now being utilized in studies on thyroiditis, in studies on alcoholic patients, especially those with evidence of thyroid hypofunction.

Richter (15), on the other hand, has taken a somewhat different approach to the problem, postulating a defect in either production or release of TSH. He cites experiments which suggest that thyroid deficiency might result from permanent damage to the thyrotropic cells of the pituitary or to the thyroid-stimulating cells of the hypothalamus. He likewise proposes the concept that man's difficulties with alcohol may result from his failure to recognize that alcohol has a high caloric value in itself, and that, by ingesting an average amount of food in addition, he may be flooding his body with an excessive number of calories. It has been reported in this regard that, by prolonged forced feeding, animals can be made hypothyroid, presumably by means of inhibition or modification of TSH release (19).

Although it is difficult to picture the usually malnourished cirrhotic patient as suffering from the consequences of overnutrition, it is nevertheless possible that such a mechanism may be etiologically significant in

the early stages of alcohol habituation. Table 1 demonstrates that of 10 obese alcoholics, 8 (80 per cent) were also hypothyroid.

It is of interest (in the light of the findings of some authors (4) which suggest that a relative or "low reserve" adrenal deficiency is common among alcoholics) that LT_3 may not only exert a profound effect on the enzymatic mechanisms of energy exchange and utilization, but also enhance, to some degree, the response of the adrenal glands to stimulation with ACTH (20). Thus the employment of LT_3 as therapy in alcoholics may have a dual influence by supporting or replacing thyroxine in the control of metabolic function, and by increasing the adrenal output of corticoids in response to stress.

Considering the relative safety of both thyroxine and triiodothyronine when used judiciously and in proper amounts, the use of these hormones approaches a nearly ideal method for the medical management of the alcoholic patient who proves responsive to such therapy. It is, however, to be emphasized that the prime motive of this preliminary study is not to advocate the use of thyroid as a panacea for alcoholism, but rather to contribute information regarding the uncommonly high incidence of hypothyroidism in this initial, though small, group of alcoholics. The facile correction of the hypothyroid state not only appears to be of great supportive benefit to the patient but seems also to be a logical way to prepare him both physically and mentally for achievement of the maximal benefit from psychotherapy.

Disulfiram (Antabuse) in the treatment of alcoholism has been successful in only a small, variable percentage of patients. There are additional reports which indicate that it depresses thyroid function in a manner similar to that of the antithyroid compounds (21). In fact, hypothyroidism is listed by Goodman and Gillman (22) as a contraindication to the use of disulfiram.

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THE COEXISTENCE OF PHEOCHROMOCYTOMA AND ADRENOCORTICAL HYPERPLASIA*

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ABSTRACT

A case of hirsutism, acne, polydipsia, polyuria, weight loss and one known episode of hypertension is reported. The patient was a 22-year-old woman. Laboratory investigations revealed glycosuria, hyperglycemia, hypermetabolism, and elevated levels of urinary catechol amines, urinary 17-ketosteroids and plasma 17-hydroxycorticoids. Upon x-ray examination, a left suprarenal mass could be seen. At operation a large benign pheochromocytoma was found in the left adrenal and also marked adrenocortical hyperplasia on the left. Nine months after surgery, the hirsutism and acne were improved. Glucose tolerance, the basal metabolic rate, and the results of the histamine test and the adrenocortical function studies were all entirely normal. Since the patient did not require cortisone therapy for a significant period of time postoperatively, and since unilateral adrenalectomy had been curative, it was inferred that the contralateral adrenal cortex at the time of operation was neither atrophic nor affected by the usual type of adrenal hyperplasia. It is postulated that both adrenal cortices were hyperplastic, secondary to some substance or substances, the source of which was removed by unilateral adrenalectomy. The older medical literature suggests that epinephrine stimulates adrenocortical function, but newer adrenocortical function tests indicate no significant effect. Therefore, there is no good evidence that the co-existence of pheochromocytoma and adrenocortical hyperplasia in this patient was other than coincidental.

THE coexistence of pheochromocytoma and proved adrenocortical hyperfunction is rare. This case is presented and discussed because: 1) it is unusual, 2) it illustrates the diagnostic value of the newer adrenal function tests, and 3) it involves important implications concerning adrenocortical-medullary relationships.

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CASE REPORT

C. B., a 22-year-old single white female was admitted to the Roanoke Memorial Hospital in March 1956, because of a skin rash, hirsutism, polydipsia and polyuria, nervousness, and emotional lability. She had been seen by her physician about one year previously because of mild menstrual irregularity, constipation, and hirsutism of one to two years' duration. At this time, the blood pressure and other findings on physical examination were normal, except for mild facial hirsutism. The hematocrit reading and BMR were normal, as were the results of urinalysis. About two months before hospitalization the patient had been seen because of episodes of nausea and a sensation of abdominal fullness. X-ray examination of the gall bladder, upper gastro-intestinal tract, colon and rectum (barium enema) had revealed nothing abnormal. One month prior to admission, the patient noted excessive thirst and urinary frequency, with a rather poor appetite, and weight loss. She had become more nervous. About one week prior to admission, she had noted an acneiform eruption on the face and shoulders. The menses had returned to normal. The past history and review of systems were otherwise noncontributory.

Physical examination revealed an apprehensive, timid, rather withdrawn white female with a blood pressure of 120/70 mm. Hg and a pulse of 76. She was well developed and nourished with normal body configuration and musculature. There was no evidence of weight loss though her medical record indicated a 12-pound loss in the previous year. There was fairly marked hirsutism of the face and definitely increased hair growth over the arms, axillae and pubic area. A fine acneiform eruption was present on the face and shoulders (Fig. 1a). The remainder of the findings on physical examination were considered normal.

Laboratory studies showed a normal urine and a hematocrit reading of 44 per cent. The white blood cell count was 27,000 and 18,000 per cu. mm., with a marked shift to the left on two determinations. The serum potassium level was 2.6 and 2.8 mEq. per liter on two determinations. The level of serum sodium was 138 mEq. per liter, chlorides 88 mEq. per liter, and CO_2 combining power 32 mMol. per liter. The blood urea level was 46 mg. per 100 ml. A glucose tolerance test (Fig. 2, preoperative data) revealed a diabetic type of curve, with glycosuria in all but the final urine specimen. The BMR was +35 per cent. An electrocardiogram showed tachycardia and a pattern suggesting left ventricular hypertrophy. Urinary 17-ketosteroid excretion was 47.5 mg. per twenty-four hours (Fig. 3, stippled bar). The plasma 17-hydroxycorticoid level was 216 μg. per 100 ml. The determination was repeated before intravenous ACTH stimulation, and a level of 212 μg. per 100 ml. was obtained. After four hours of ACTH stimulation (Fig. 4, preoperative curve) the value was 207 μg. per 100 ml. Urinary catechol amine excretion was 30 μg. per hour (normal, 1.6–6.6 μg.) calculated as norepinephrine and determined[1] by the method of Helmer and Sanders (1). An intravenous urogram revealed a left suprarenal mass about 5 cm. in diameter.

During the *preoperative course*, the temperature was normal and the pulse ranged from 70 to 120 beats per minute. The blood pressure was normal except for an occasional rise to 140–150/90–100. However, on the third and again on the fourth hospital day, the patient experienced an episode of increased nervousness, excitement and irritability, and complained of diffuse abdominal pain and a sensation of numbness in her arms and

[1] Determinations kindly performed for us by Dr. Oscar M. Helmer of the Lilly Laboratory for Clinical Research, Indianapolis, Indiana. (This method does not determine the relative amounts of epinephrine and norepinephrine.)

a b

FIG. 1. Appearance of patient **a**) before, and **b**) after unilateral adrenalectomy.

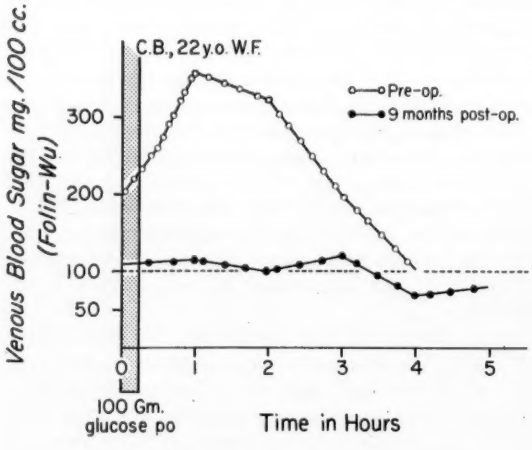


FIG. 2. Oral glucose tolerance curve before and after unilateral adrenalectomy.

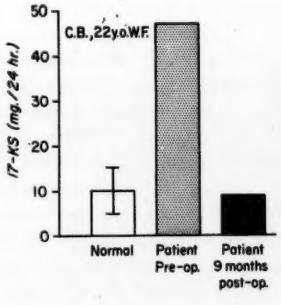


FIG. 3. Urinary 17-ketosteroid excretion before and after unilateral adrenalectomy.

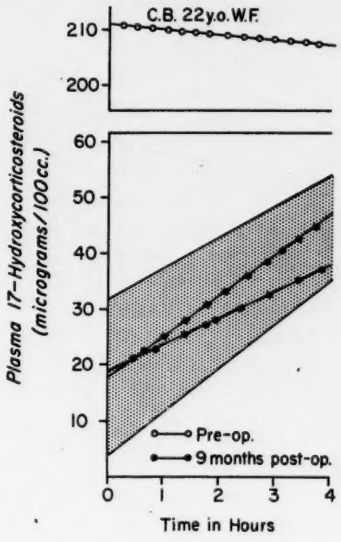
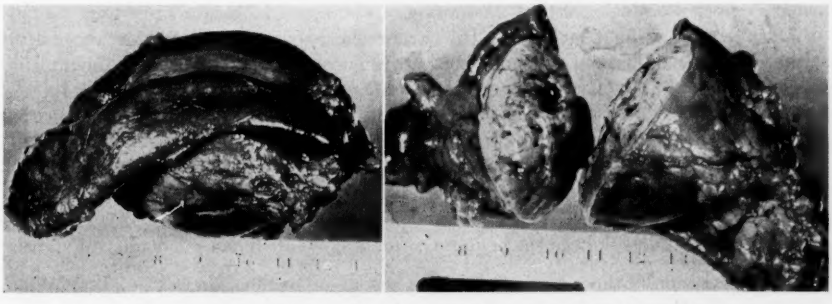


FIG. 4. Response of plasma 17-hydroxycorticoid levels to exogenous ACTH stimulation before and after unilateral adrenalectomy. Note the increase on the ordinate, and the normal range as depicted by the stippled band.

legs. These symptoms improved after one or two hours, with sedation. She was examined during the second attack and the blood pressure was found to be 180/120 with a pulse of 100; no other abnormalities were observed at that time. It was this episode that suggested the possibility of pheochromocytoma and prompted the determination of urinary catechol amines. The result of the assay was not known, however, at the time of surgery.

Operation. On the basis of the findings, it was thought the patient had an adrenocortical tumor on the left and that she possibly also had a pheochromocytoma. On the ninth hospital day, operation was performed (by W. W. S. B.) through a thoraco-abdominal approach. In the left suprarenal area there was a diffusely enlarged adrenal, with a large spherical tumor on its inferior aspect. The entire gland and the tumor were removed. Examination of the specimen (Figs. 5 & 6) revealed a large adrenal weighing 60 Gm. and measuring 8.5×4×1.5 cm. The spherical mass weighed 40 Gm., was 4 cm. in diameter,



FIGS. 5 and 6. Left adrenal gland, showing pheochromocytoma and hyperplastic adrenal cortex.

FIG. 7. Histologic features of the pheochromocytoma. (H&E, 225×)

and exhibited a positive chromic-acid reaction. Microscopic examination[2] showed that this tumor was a benign pheochromocytoma (Fig. 7). The cortex of the adrenal gland weighed 20 Gm. and was hyperplastic (Fig. 8).

The patient was given an infusion of levo-arterenol, 4 mg. per 1000 ml. of dextrose in water, during operation and for the first eighteen hours thereafter. Except for a short period of profound hypotension when the adrenal vessels were clamped—a condition which was initially difficult to relieve—the blood pressure was well maintained. The patient received 100 mg. of cortisone intramuscularly the night before surgery, 350 mg. in fractional doses on the day of surgery, 75 mg. on the first postoperative day, 12.5 mg. daily on the second to fourth days, and none thereafter. There was no evidence of adrenal insufficiency and she made an uneventful recovery. On the tenth postoperative day, the urinary catechol amine excretion was normal.

Progress notes. Nine months later, the patient was hospitalized in the Metabolic Unit at the University of Virginia Hospital for re-evaluation. She had felt well in the interim. She was somewhat less nervous, her appetite had returned, there were no gastro-intestinal symptoms and her weight had returned to its usual level. She had no polydipsia or polyuria. The hirsutism and acne were much improved and menses were regular.

She was still somewhat withdrawn emotionally. The pulse was 100 and the blood pressure 115/70. She had obviously gained weight but her general body configuration and musculature were normal. Hirsutism and acne were now minimal (Fig. 1b).

Laboratory studies showed the hematocrit reading, white blood cell count, differential

[2] Interpretation of the pathologic findings was made by Dr. James C. Gale, Pathologist, Roanoke Memorial Hospital, Roanoke, Virginia.

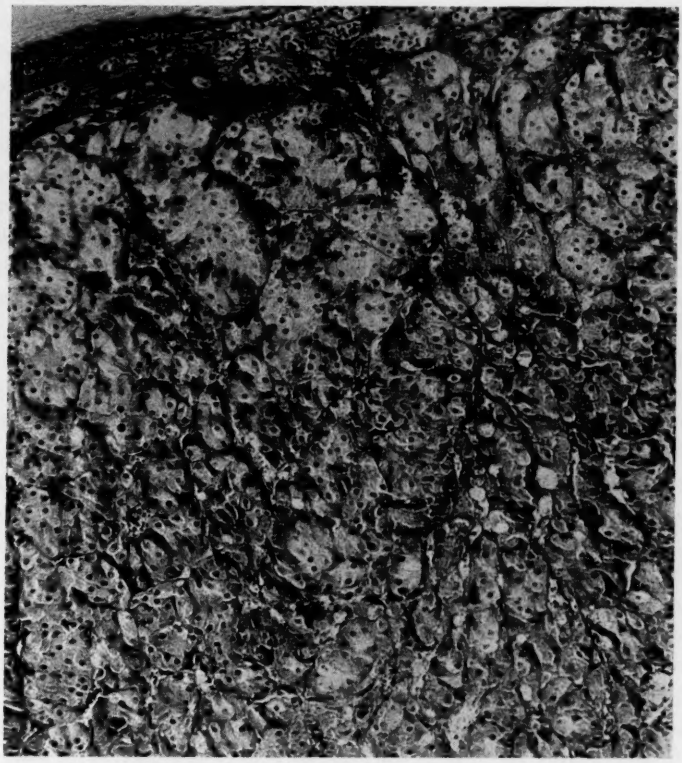


FIG. 8. Histologic features of the hyperplastic adrenal cortex. (H&E, 130×)

count, and levels of serum electrolytes to be normal. In the electrocardiogram there were low T waves but no evidence of left ventricular hypertrophy. The BMR had fallen from +35 per cent before surgery to −11 per cent. Urinary catechol amines were not determined, but results of the histamine test were normal. The glucose tolerance curve, which was of a diabetic type preoperatively, was now flat (Fig. 2, lower curve). Urinary 17-ketosteroid excretion, which had been markedly elevated preoperatively, had fallen to normal levels (Fig. 3, black bar). The concentration of plasma 17-hydroxycorticoids, which had been markedly elevated and unresponsive to ACTH preoperatively, was now normal and responded normally to ACTH stimulation on two occasions (Fig. 4, lower curves).

DISCUSSION

A review of the medical literature revealed several reported cases with at least suggestive evidence of co-existing abnormalities of the adrenal medulla and cortex (2–16). The findings in these cases varied widely. Robinson and Baker-Bates (2) reported a patient with symptoms of nine

years' duration, strongly suggesting pheochromocytoma. However, at operation an adrenocortical carcinoma with metastases was found. There was no evidence of pheochromocytoma histologically or by staining and assay tests. The patient died during operation, following a rise in blood pressure to unmeasurable levels. Conversely, Neff *et al.* (3) reported a case of a 16-month-old female with hypertension and clinical evidence of both Cushing's syndrome and the adrenogenital syndrome. However, at operation only a pheochromocytoma was found, with no histologic evidence of cortical abnormality. Within three months after surgical removal of the tumor, the patient was clinically normal. Mulrow and Cohn (4) recently observed a patient from whom a large adrenal tumor was removed. Histologically the tumor was a pheochromocytoma and large amounts of catechol amines were extracted from it. However, upon *in vitro* incubation, this tumor also produced a substance indistinguishable from cortisol. These authors suggested that histologic criteria alone may be inadequate for assessment of the pathologic functioning of adrenal tumors. The clinical status and laboratory determination of their patient's adrenocortical function were not reported. Cope *et al.* (5) observed a patient with clinical evidence of a pheochromocytoma, in whom a pheochromocytoma and an adrenocortical adenoma were found in the same adrenal at operation. In view of the fact that the remaining adrenal cortex was atrophied and there was postoperative adrenocortical insufficiency, it was concluded that the cortical adenoma was functioning. However, there was no clinical or laboratory evidence of hyperadrenocorticism preoperatively, and the urinary 17-ketosteroid excretion was low.

Reported studies of adrenocortical function in other patients with known pheochromocytoma are few and conflicting. Urinary 17-ketosteroid excretion has been reported as normal (6), elevated (7), and low (5, 8). Urinary 17-hydroxycorticoid excretion has been reported as elevated (7), elevated with no response to ACTH stimulation (8), and elevated with hyper-responsiveness to ACTH stimulation (9). In addition to a greatly elevated excretion of catechol amines, our patient had a markedly elevated excretion of urinary 17-ketosteroids and a very high level of plasma 17-hydroxycorticoids which did not rise further upon stimulation with ACTH. These steroid values are more indicative of adrenocortical carcinoma than of hyperplasia.

The case reported here is also unique in that preoperative studies revealed definite evidence of both pheochromocytoma and adrenocortical hyperfunction, whereas studies repeated nine months after surgery revealed normal function in both respects. Moreover, at operation, histologic evidence of both pheochromocytoma and adrenocortical hyperplasia

was found in the left adrenal. The status of the opposite adrenal at the time of surgery is not known, as the precarious condition of the patient prevented exploration of this area. All that can be stated from our data is that nine months after surgery there was clinical and laboratory evidence of normal function in the remaining adrenal. One can draw certain inferences, however. The amount and duration of postoperative cortisone therapy were less than generally required following removal of a unilateral hyperfunctioning adrenocortical lesion with contralateral cortical atrophy. In our experience (17) and that of others (18), unilateral adrenalectomy does not cure hyperadrenocorticism due to bilateral adrenal hyperplasia. Therefore, it is considered unlikely that the right adrenal cortex was atrophic, or that it was affected by the usual type of pathologic adrenal hyperplasia at the time of surgery.

It is interesting to speculate as to whether there was any direct relationship between the pheochromocytoma and the cortical hyperplasia, or whether the two lesions were only coincidental. Based on the foregoing data and inferences, an attractive hypothesis would be that both adrenal cortices were being overstimulated by some substance or substances, the source of which was removed at operation, and that following this removal, the remaining adrenal returned to its normal functional state. At the time of their report in 1952 Cope *et al.* (5) postulated that excess epinephrine secretion from the pheochromocytoma could have induced hyperplasia of the adrenal cortex by stimulating release of ACTH from the pituitary. The evidence in our case, and the foregoing hypothesis are compatible with this theory. However, experimental supporting data are very tenuous.

The work of several investigators in the past has suggested that epinephrine is capable of stimulating the adrenal cortex, either directly (19, 20), or by stimulating the anterior pituitary to secrete ACTH (21, 22). This evidence was based in most cases on the development of changes resembling adrenocortical effects, rather than on direct measurements of ACTH or adrenal hormones. More recent experimental evidence indicates that chronic administration of epinephrine will not produce adrenocortical hyperplasia or hyperfunction (23), and that administration of epinephrine does not result in a significant increase in the urinary excretion of 17-hydroxycorticoids and 17-ketosteroids (24, 25) nor in the plasma levels of 17-hydroxycorticoids (25).

The available data from the literature therefore do not indicate a significant effect of epinephrine on the control of adrenocortical function and do not support a causal relationship between lesions of the adrenal medulla and lesions of the adrenal cortex.

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CUSHING'S SYNDROME WITH MULTINODULAR ADRENAL GLANDS*

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ABSTRACT

A case of Cushing's syndrome in a 15-year-old girl with symptoms of one year's duration, is described. The levels of plasma and urinary 17-hydroxycorticosteroids and urinary 17-ketogenic steroids were elevated but the levels of urinary 17-ketosteroids and pregnanetriol were normal. Stimulation with ACTH produced a low, sluggish response in the concentration of plasma 17-hydroxycorticosteroids and only minimal changes in urinary steroids. Administration of 9α-fluorohydrocortisone produced no change in urinary steroid excretion. Following bilateral adrenalectomy the manifestations of Cushing's syndrome disappeared. The adrenals showed diffuse multinodularity of the inner cortex. The relation of this lesion to other adrenal lesions producing Cushing's syndrome is discussed.

MELLINGER and Smith in 1956 reported an unusual case of Cushing's syndrome of about thirty years' duration in a patient who had multiple small nodules throughout the cortex of one adrenal. Biopsy of the same gland earlier in the course of the disease had not shown this lesion (1). The contralateral gland was not atrophic and did not show the nodular change. The nodular adrenal pattern was believed to be peculiar to very long-standing Cushing's syndrome.

Cohen *et al.* recently presented evidence that nodularity exists to a greater or less extent in all cases of adrenal hyperplasia, dependent upon the intensity of adrenal stimulation (2). In their material, there was no cytologic difference between the nodular tissue and adenomatous tissue. Unfortunately, the paucity of combined biochemical and pathologic observations in Cushing's syndrome limits the clinical application of purely pathologic data.

The case reported here showed, in contrast to the case of Mellinger and Smith, multiple nodularity of the adrenals in Cushing's syndrome of rela-

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tively short duration. Biochemical data after attempted stimulation and suppression of the adrenals were atypical for adrenal hyperplasia.

CASE REPORT

J.E.W. (UCLA 014-37-27), a 15-year-old white girl, was referred to the University of California Hospital at Los Angeles on September 17, 1957, because of severe back pain of five months' duration. Three years prior to admission, the patient began menstruating and had undergone the normal pubertal growth spurt. The development of the breasts and sexual hair was normal for her age.

One year before admission the menses ceased. Increasing fullness of the face, increased general body and facial hair and mild chronic irritabiltiy developed. Five months prior to admission, there was an abrupt onset of back pain, which became progressively worse. During the month prior to admission, a pronounced increase occurred in the fullness and plethora of the face, truncal obesity, easy bruising and poor healing of minor skin injuries. Wide, purplish striae began to appear over the hips, breasts and abdomen.

Physical examination. Temperature 37.2° C., pulse 88, respirations 22, blood pressure 140/80 mm. Hg, height 142 cm., weight 42.6 Kg. The patient was a well developed white girl with marked truncal obesity and somewhat thin, spindly legs. There was a heavy fat pad in the interscapular area of the back. The face was round, due to heavy temporal padding. The skin was warm and moist. The cheeks were flushed and there was moderate seborrhea and acne. Broad purple striae were present over the lateral aspects of the abdomen and breasts. There were numerous ecchymoses on the arms and legs and several old, unhealed superficial scratches on the legs. Excessive hair was present on the upper lip and over the trunk and extremities. There was exquisite tenderness and muscle spasm in the region of the twelfth thoracic vertebra. Ophthalmoscopy and visual field perimetry showed no defect. The patient's appearance at the time of admission is shown in Figure 1.

Laboratory data on admission. Hemoglobin 14.5 Gm. per 100 ml.; white blood cell count, 12,000 per cu. mm. with 74 per cent neutrophils, 24 per cent lymphocytes and 1 per cent monocytes; platelet count, 417,000 per cu. mm.; bleeding time, one and a half minutes; and clotting time, four and a half minutes in capillary tubes. Routine urinalysis: urine light yellow and slightly cloudy with a specific gravity of 1.008; pH 7.5; no protein, sugar or acetone; and 8 to 10 erythrocytes, 1 to 2 leukocytes and an occasional fine granular cast in each high-power field. Blood chemistry: concentration of fasting blood sugar 134 mg. per 100 ml., serum sodium 143 mEq. per liter, potassium 3.2 mEq. per liter, calcium 5.7 mEq. per liter, CO_2 26.7 mEq. per liter, chloride 100 mEq. per liter, phosphorus 2.0 mEq. per liter, albumen 4.1 Gm. per 100 ml., globulin 2.4 Gm. per 100 ml., alkaline phosphatase 11.3 King-Armstrong units per 100 ml., cholesterol 315 mg. per 100 ml., nonprotein nitrogen 44 mg. per 100 ml., and protein-bound iodine 7.0 μg. per 100 ml. An electrocardiogram revealed slight left ventricular preponderance which was probably normal for the patient's age. Stool specimens contained Enterobius ova.

X-ray examination of the skull showed normal configuration of the sella turcica. Views of the thoraco-lumbar spine indicated severe osteoporosis with collapse of several vertebrae. An intravenous urogram showed rapid excretion of the dye with normal renal calyceal pattern except for the presence of calculi in the pelvis of the left kidney. There was no evidence of an extra-renal tumor.

Adrenal function tests.[1] The Robinson-Power-Kepler water-loading test showed a

[1] All steroid determinations were performed by the Bio-Science Laboratories, Los Angeles 25, California. The methods used were as follows—Urinary 17-hydroxycorti-

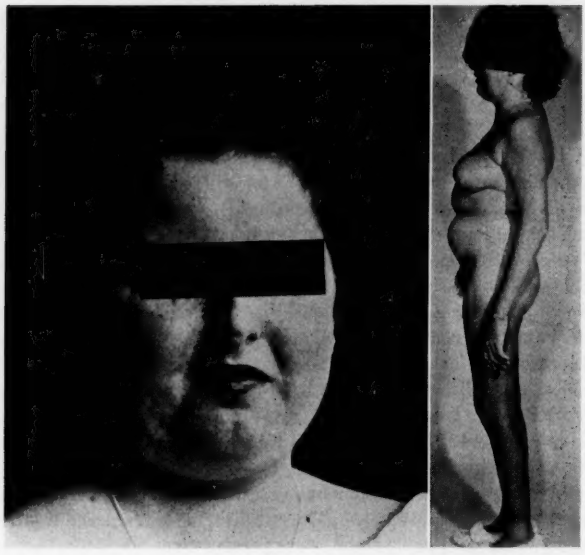

FIG. 1. The patient at the time of admission.

borderline delay in water diuresis. The responses of the urinary steroids to stimulation with ACTH and suppression with 9α-fluorohydrocortisone (9α-FF) are shown in Table 1. There was no significant change in the urinary excretion of steroids after ACTH. Plasma levels of 17-hydroxycorticosteroids (17-OH-CS) were measured during a two-hour infusion of ACTH on Day 3, and during a six-hour infusion on Day 12. On Day 3, the levels of 17-OH-CS fasting, and at two, four and six hours were 18.5, 24.5, 25.1 and 19.6 μg. per 100 ml., respectively. On Day 12, the levels fasting, and at four and six hours were 30, 38 and 42 μg. per 100 ml., respectively. Suppression with 9α-FF was not carried past the fourth day. The values on Day 4 were lower than those on Day 3, but were not below control levels.

Surgery. On October 8, 1957, after preoperative preparation of the patient with intramuscular cortisone acetate, bilateral adrenalectomy was performed by the transabdominal approach. The adrenals grossly appeared to be hemorrhagic and were removed without technical difficulty.

Postoperative course. The patient received decreasing doses of cortisone acetate, until two and a half weeks after operation she was receiving 12.5 mg. twice daily by mouth. This dosage was continued through two years of observation. Initially she received added salt each day; after several weeks an ordinary diet was maintained. Cushingoid features disappeared after operation. The patient's appearance six months after surgery is shown in Figure 2.

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costeroids (17-OH-CS): Glenn and Nelson (3), modified by a preliminary extraction of the steroid conjugates from the urine according to the procedure of Edwards *et al.* (4). Plasma 17-OH-CS: Nelson and Samuels (5). Urinary 17-ketosteroids (17-KS) and 17-ketogenic steroids (17-KGS): Sobel *et al.* (6). Urinary pregnanetriol (PT): Cox (7), modified by a preliminary extraction as in the method for 17-OH-CS.

TABLE 1. EXCRETION OF URINARY STEROIDS DURING PREOPERATIVE ADRENAL STIMULATION AND SUPPRESSION TESTS
(values expressed as mg. per 24 hours)

Day	Procedure	17-KS	17-OH-CS	17-KGS	Pregnanetriol
1	Control	9.1	10.2	—	—
2	Control	5.9	8.9	25.9	1.8
3	ACTH, 25 units i.v.*	8.2	9.3	24.9	0.9
4	ACTH, 8 units i.v.**	7.9	9.2	21.8	0.8
12	ACTH, 25 units i.v.†	13.2	11.3	34.5	2.3
6	9α-FF, 4 mg. orally‡	—	—	—	—
7	9α-FF, 4 mg. orally	—	—	—	—
8	9α-FF, 4 mg. orally	12.3	18.9	32.5	2.2
9	9α-FF, 4 mg. orally	10.0	9.1	17.4	1.8

* Two-hour infusion.
** Eight-hour infusion;
† Six-hour infusion.
‡ Dose of 2 mg. every twelve hours.

Pathology. The adrenal glands were surrounded by fatty tissue showing recent hemorrhage. Their combined weight was 16.2 Gm. (The average normal weight of both adrenals combined, at this age, is approximately 10 Gm.) Multiple sections showed normal cortical configuration. Throughout the pale yellow cortex in all sections there were many greenish-brown nodules which were entirely confined within the cortical ribbon (Fig. 3). No nodules were present outside the cortical capsule.

FIG. 2. The patient, six months after bilateral adrenalectomy.

FIG. 3. Low-power view of adrenal gland showing configuration of cortical nodules (silver impregnation, Warthin-Starry, 40×).

Microscopically, the cortical architecture was distorted by nodular cellular masses occupying a segment or the entire thickness of the cortex. The nodules were not encapsulated and were composed of polyhedral or irregular cells much larger than surrounding normal cortical cells. The cells were irregularly arranged in the nodules, or tended to form cords or strands separated by fine capillary or connective tissue. The cells varied considerably in size and contained lightly eosinophilic and granular cytoplasm which varied from solid to foamy or vacuolated. The nuclei were rounded and vesicular. Although there were frequent giant nuclei there were no other bizarre nuclear forms. Numerous cells contained fine, brown pigment granules with occasional coalescence reminiscent of the pigmented cells of the zona reticularis. The cells of the surrounding cortex were typ-

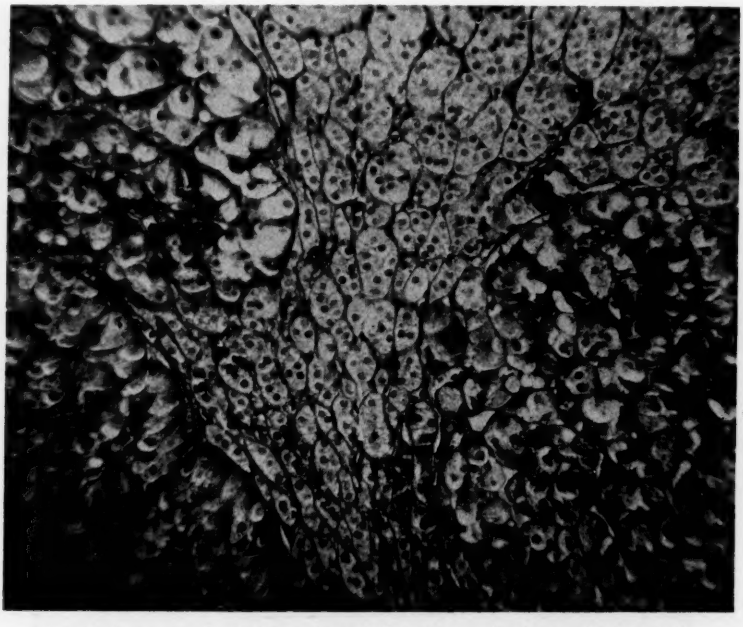

FIG. 4. Appearance of tissue in multiple hyperplastic cortical nodules separated by normal cortical cells (Masson trichrome, 100×).

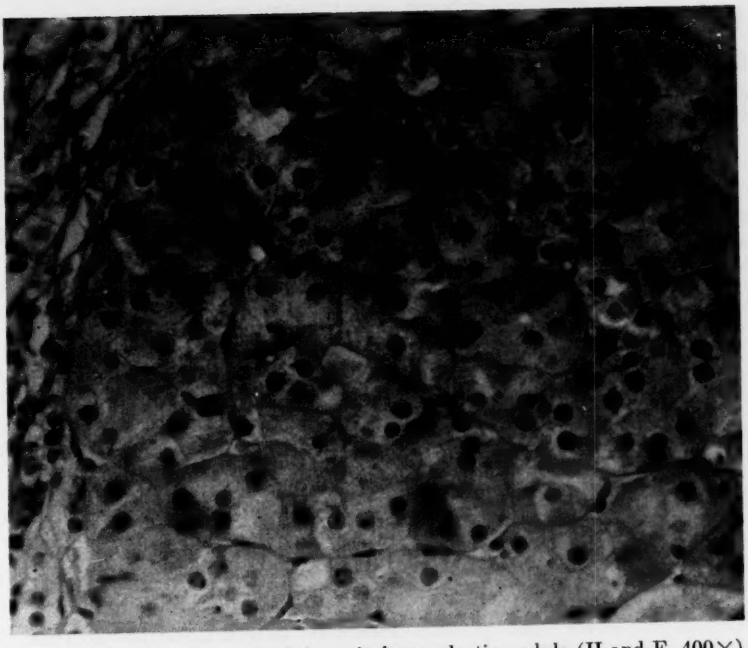

FIG. 5. Typical appearance of tissue in hyperplastic nodule (H and E, 400×).

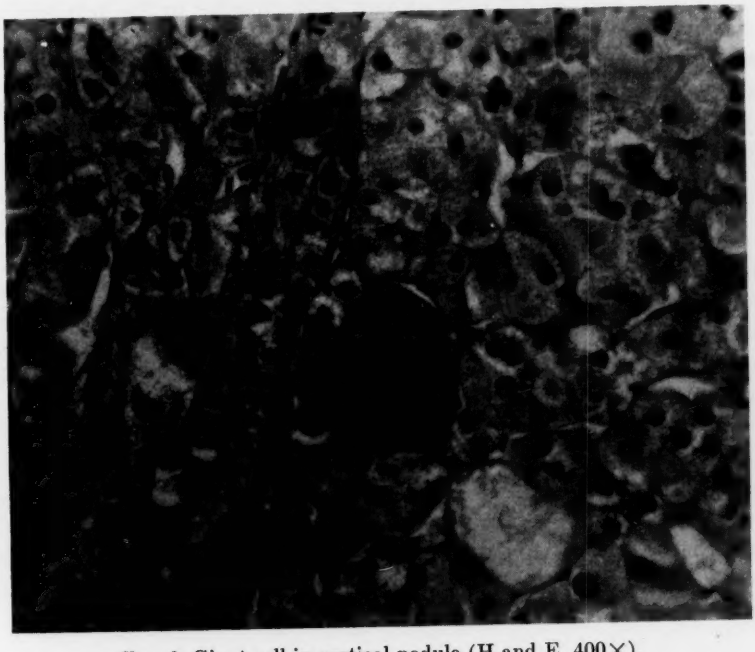

FIG. 6. Giant cell in cortical nodule (H and E, 400×).

ical in morphology and structural organization except for focal atrophy, perhaps due to compression, around the hyperplastic nodules. Severe vascular congestion and minute hemorrhages appeared to be related to the surgical procedure (Figs. 4, 5 and 6). The zona glomerulosa was not only generally thin, but where it was adjacent to the nodules, it too was compressed, fibrosed and atrophic, or absent. The changes in the glands were similar, in most respects, to those described in a recent study by Cohen *et al.* (2) of the characteristics of resected adrenal glands in Cushing's disease. These authors found that the lipid content of the nodular cells varied considerably. The nodules present in the adrenals of our patient tended to resemble the large lipid-poor nodules which Cohen *et al.* describe.

DISCUSSION

Cushing's syndrome is usually easily recognized. However, there can be considerable doubt as to whether the cause is adrenal hyperplasia, adenoma or carcinoma. Stimulation and suppression tests may aid in the differentiation of these lesions. The hyperplastic adrenal causing Cushing's syndrome is likely to be hyperresponsive to stimulation with ACTH and to be suppressed by intensive administration of exogenous corticoid. An adrenal adenoma is less predictable in its reaction, and an adrenal carcinoma usually fails to respond to either stimulation or suppression (8–11).

There is evidence that plasma levels of dehydroisoandrosterone, androsterone and etiocholanolone are higher in the type of Cushing's syndrome due to adrenal hyperplasia and that changes in the levels of androsterone and etiocholanolone after adrenal suppression or stimulation differ between adrenal hyperplasia and adenoma (12). The fraction of the urine containing dehydriosoandrosterone and isoandrosterone may also be relatively high when Cushing's syndrome is due to adrenal hyperplasia (13). A high baseline excretion of urinary 17-ketosteroids has been correlated with adrenal carcinoma (14).

The infusion of ACTH in our patient produced only a mild increase in the plasma level of 17-hydroxycorticosteroids (17-OH-CS) and had little or no effect on urinary 17-ketosteroids (17-KS), 17-ketogenic steroids (17-KGS), or 17-OH-CS. This pattern would ordinarily be considered suggestive of an autonomous adrenal lesion, such as carcinoma or adenoma The results with ACTH indicate that the multinodular lesion in this case either functioned independently of pituitary control, or was already secreting corticosteroids at a maximal rate.

The lack of suppression of adrenal steroid production in our patient after administration of 4 mg. of 9α-FF daily may not be conclusive. Liddle reported that 2 mg. daily of Δ^1-9α-FF or its 16α-methylated analog, dexamethasone, failed to suppress 17-OH-CS excretion in cases of Cushing's syndrome due to hyperplasia, but that 8 mg. daily produced definite decreases in all cases (15).

Cohen *et al.* (2) suggest that the multinodular adrenal develops as a response to extreme stimulation. The case of Mellinger and Smith (1) provides evidence that mild protracted stimulation can also produce this effect. Our observations indicate that the multinodular adrenal may lose its responsiveness to ACTH and function autonomously like a typical neoplasm. The patient of Mellinger and Smith resembled our patient in having only a moderately elevated excretion of 17-OH-CS and a normal excretion of 17-KS but she had a greater response to administration of ACTH. This difference may only represent less regression toward an autonomous state.

Whether or not the multinodular adrenal is an adrenal neoplasm would seem a moot point, although Cohen *et al.* could find no morphologic or histochemical features distinguishing intracortical nodules from adenomata. More detailed identification and quantitation of steroids, and correlation with the adrenal pathology in Cushing's syndrome may throw additional light on the relationship between the multinodular adrenal and adrenal neoplasm.

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AMENORRHEA AND ELEVATED LEVEL OF SERUM CHOLESTEROL PRODUCED BY A TRIFLUOROMETHYLATED PHENOTHIAZINE (SKF 5354-A)

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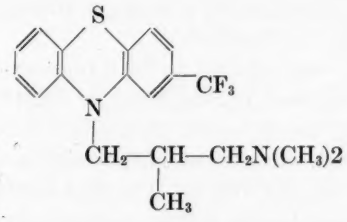
ABSTRACT

The administration of 1 Gm. per day of a trifluorinated phenothiazine (SKF 5354-A) resulted in amenorrhea in 6 of 7 female patients, without a rise in urinary gonadotropin excretion. The drug also produced a rise in the serum cholesterol level. There was a slight depression of 24-hour thyroidal I^{131} uptake, but not sufficient to account for the increase in serum cholesterol concentration.

THE effects of several phrenotropic agents, particularly chlorpromazine (CPZ), on adrenal, thyroidal and gonadal function have been reported by several investigators (1–18) although the precise mechanisms involved have not been clearly elucidated. Most authors favor inhibiton of pituitary function by the hypothalamus, whereas Reiss (6) takes the view that CPZ inhibits the target glands directly, resulting first in stimulation and then in exhaustion of the pituitary. Suzuki *et al.* (5) offer evidence that CPZ exerts both a central and peripheral depressant effect on the endocrine system. Since more information will be needed to clarify this problem it is deemed worth while to report our recent experiences with a new phenothiazine preparation, 10(3-dimethylamino-2-methylpropyl)-2-trifluoromethyl-phenothiazine hydrochloride. The code name is SKF 5354-A.



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[1] Hycel hormone chemistry method, Hycel Hormone Chemistry Laboratory, Houston, Texas.

Our attention was first directed to the endocrine effect of this drug during a study of the dose range, using 3 male and 3 female psychiatric patients. In the 3 women, as the daily dose was gradually increased from 15 mg., amenorrhea without galactorrhea developed. This first occurred when a dosage of approximately 1.0 Gm. per day was reached. Unfortunately no pretreatment endocrine data had been obtained. Confirmation of this interesting finding was then undertaken.

PROCEDURE

Group 1. After the onset of amenorrhea in the 3 female patients, laboratory data pertinent to possible acquired endocrine dysfunction were obtained on them and also on the 3 male patients. These data included thyroidal I^{131} uptake, serum protein-bound iodine level, serum cholesterol level, and urinary gonadotropin excretion. These determinations were repeated the first and second months after discontinuing the drug.

Group 2. A second group of 4 female chronic schizophrenic subjects, aged 33 to 39, was selected on the basis of a known regular menstrual history. These women exhibited no recent change in the psychiatric disease, and physical examination and routine laboratory work showed that they were in good physical condition. They were housed on the medical ward for this study and were active and participated in all ward activities. Menstrual periods were recorded through three cycles, and found to be regular in 3 subjects; in Patient B.K. the third menstrual period was delayed ten days and fell within the first month of drug administration. The following laboratory studies were carried out at intervals prior to, during, and after medication: vaginal smears (19), urinary gonadotropin (20), serum protein-bound iodine level,[1] 24-hour thyroidal I^{131} uptake, and serum cholesterol level (21). In addition, special attention was paid to the accurate recording of the menstrual cycle, body weight, and daily basal body temperature.

Medication with SKF 5354-A was started as a small oral dose which was raised by increments over a thirty-day period to a level of 1 Gm. per day. This dosage was continued for one month and then decreased stepwise, and stopped during the next month. Observations were continued for five months after all medication had been stopped. The dose used in this study is greater than that which is ordinarily considered adequate for a therapeutic effect.

RESULTS

The results by months for each subject are recorded in Tables 1 and 2. When more than one determination was made during a month, the average value for that month is reported.

Group 1. Amenorrhea was induced in the 3 females during therapy, and normal menstruation returned by the third or fourth month after treatment was stopped. The mean serum cholesterol level was 282 mg. per 100 ml. during treatment, and in the post-treatment period it decreased to a mean level of 156 mg. per 100 ml. The 24-hour thyroidal I^{131} uptake, the serum level of protein-bound iodine, and urinary gonadotropins were within normal limits throughout.

Group 2. In 3 of the 4 subjects amenorrhea developed during the second month of medication; it persisted for three months after therapy had been discontinued. Metrorrhagia developed in Patient H.K. during treatment.

TABLE 1. SUPPRESSION OF MENSTRUATION BY A TRIFLUORINATED PHENOTHIAZINE DERIVATIVE GIVEN TO 7 CHRONICALLY ILL PSYCHIATRIC PATIENTS*

Patients	Pre-treatment period			Treatment period			Post-treatment period				
	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 4	Month 5
Group 1											
B.C.					0	0	0	0	+	+	?
M.V.					0	0	0	0	0	+	?
M.D.					0	0	0	0	0	+	+
Group 2											
B.K.	+	+	0	+	0	0	0	0	0	+	+
K.D.	+	+	+	+	0	0	0	0	+	+	+
N.S.	+	+	+	+	0	0	0	0	+	+	+
H.K.†	+	+	+	+	++	++	0	+	+	+	+

* A plus sign indicates a normal menstrual period. Zero indicates no menstruation. A dosage of 1 Gm. per day was given during the second month of treatment.

† Patient H.K. had metrorrhagia throughout the second and third months of treatment.

TABLE 2. EFFECT OF A TRIFLUORINATED PHENOTHIAZINE DERIVATIVE ON THE SERUM CHOLESTEROL LEVEL (MG./100 ML.) AND THYROIDAL I^{131} UPTAKE (% DOSE)

	Pretreatment period			Treatment period			Post-treatment period				
	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 4	Month 5
Serum cholesteral level (normal 150–250 mg./100 ml.)											
Group 1											
Female											
B.C.						310	198	168			
M.V.						296	190	150			
M.D.						253	180	139			
Male											
J.C.						296	156	156			
F.M.						210	180	163			
A.L.						327	156	158			
Mean						*282*†	*178*†	*156*†			
Group 2											
Female											
B.K.			292*	313	318*	332*	333*			267	285
K.D.			239*	226	212*	262*	251*			246	228
N.S.			181*	210	224*	249*	241*			202	193
H.K.			253*	226	276*	341*	242*			227	263
Mean			*241*†	*244*	*258*	*296*†	*267*†			*236*†	*242*†
24-Hour thyroidal I^{131} uptake (normal 5–20%)											
Group 1						13.1±0.9		11.1±1.7			
Group 2	15.5±0.6†			17±0.7	12.5±0.6†		17.3±1.2			17±1.6	

* Means of two or more determinations.

† Significant statistically, ($P < .05$).

After medication was stopped, normal menstrual cycles returned.

In all subjects of Group 2 there was a rise in the serum cholesterol level—mean value, 296 mg. per 100 ml.—during therapy and a return to pretreatment levels when the medication was stopped. This rise in the serum cholesterol level was of significance statistically ($P < .05$) by the third treatment month.

Whereas the concentration of serum protein-bound iodine varied randomly within the normal range throughout the observation periods, the thyroidal I^{131} uptake (normal, 5–20 per cent) decreased slightly (mean, 12.5 per cent) in all subjects during peak dosage and returned to the pretreatment level in the post-treatment period. Although this change occurred within the normal range for I^{131} uptake, it was significant statistically ($P < .05$) when compared to the pretreatment mean.

During therapy with SKF 5354-A, urinary gonadotropin excretion remained normal, and no rise occurred in any of the subjects. Rather, there was a tendency toward low values during and following therapy.

Estrogenic activity, as demonstrated by vaginal smears, was little affected by administration of the drug. Only 1 subject (B.K.) exhibited suppression of estrogenic activity during the drug period, and there was a return to normal three months after treatment was stopped. The basal body temperature, taken rectally before rising in the morning throughout the observation periods, followed no particular pattern. During therapy, 2 patients lost weight—H.K., 9 pounds, and N.S., 11 pounds. Patient B.K. gained 6 pounds and Patient K.D. showed no change in weight. After the drug was discontinued, body weight remained stable in 3 subjects, but Patient H.K. lost an additional 10 pounds over four months. Skin photosensitivity developed in all subjects at a dosage level of 1.0 Gm. per day. A Parkinson-like tremor was noted in 2 patients (N.S. and H.K.). Minor hypotensive episodes and soporific effects were noted only during the early part of treatment. These were of little clinical significance and required no specific therapy. Serial blood counts, urinalysis and liver function studies showed no changes. Galactorrhea was not noted.

DISCUSSION

Although the agent used in this study was not chlorpromazine, its analogous structure provided a reasonable basis to assume that it would act similarly. The most striking observations during treatment with this new drug were the disturbances of the menstrual cycle, and the rise in the serum cholesterol level.

The fact that there was no rise in urinary gondaotropin excretion suggests a pituitary or hypothalamic cause for the amenorrhea. Many investigators (9, 10, 12–16, 18) suggest such a mechanism for chlorpromazine. The transient early rise in gonadotropin excretion reported by others (6,

9, 10, 13, 14) was not demonstrable in our series, although determinations were made three and six weeks after starting treatment.

Other investigators (1–3) have presented evidence that the adrenal response to stress is suppressed by chlorpromazine, whereas the adrenal response to exogenous ACTH is not. We were unable to study the adrenal effect of SKF 5354-A because excretion of the drug in the urine interfered with the determination of urinary steroids.

Milcou *et al.* (7) reported that in rats chlorpromazine caused a reduction in thyroidal I^{131} uptake, a decrease in metabolic rate and a decrease in the responses to thyroid and thyrotropic hormone. Clinical improvement in hyperthyroidism was also reported with chlorpromazine. On the other hand, it has been shown in man that 200 mg. of promazine daily does not affect the thyroidal I^{131} uptake nor the serum levels of protein-bound iodine and cholesterol (8). In our series there was only a slight diminution of thyroidal I^{131} uptake, no change in the level of serum protein-bound iodine, and no clinical signs of hypothyroidism.

In considering the many conflicting reports and the apparent generalized and widespread endocrine effects of chlorpromazine, it would seem attractive to accept the idea of a direct chlorpromazine action on the pituitary or hypothalamus. The possibility of additional action on the target endocrine glands is yet to be ruled out.

The rise in the serum cholesterol level is unexplained. The slight diminution of thyroidal I^{131} uptake would not explain the marked change in cholesterol concentration, nor would variation in diet, body weight or physical activity be sufficiently great to account for it. Wilens *et al.* (22) reported that chlorpromazine is effective in reducing the serum cholesterol and phospholipid levels of cholesterol-fed rabbits, with a resulting decrease in arterial lipid deposition. They also noted a transient increase in the serum cholesterol and phospholipid levels of the control group of rabbits which received chlorpromazine without additional cholesterol in the diet. In view of the increased serum cholesterol levels sometimes associated with chlorpromazine jaundice, they suggested the possibility that the alteration in cholesterol metabolism originates in the liver.

Acknowledgments

The authors are indebted to Dr. R. Palmer Howard, Oklahoma Medical Research Foundation, for determinations of urinary gonadotropin and help in interpreting these data, and to Dr. F. Alexander, Smith, Kline and French Laboratories, Philadelphia, for generous supplies of the drug.

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Letter to the Editor

FREE ESTRIOL IN BLOOD PLASMA*

To the Editor:

Veldhuis (1), Nakao and Aizawa (2), Varangot (3), Aitken and Preedy (4), Diczfalusy and Lindkvist (5) and Slaunwhite and Sandberg (6) have described chemical methods for the estimation of estrone, estradiol-17β and estriol in blood and plasma. In some studies the free compounds were estimated and in others, hydrolytic procedures were performed prior to extraction, purification and estimation. In these reports (excepting that of Slaunwhite and Sandberg who used hydrolysis) definite criteria were not presented for the identity of the final fraction assayed as estrone, estradiol-17β, or estriol. The present communication presents definitive evidence for the presence of "free" estriol in the plasma of women at the time of delivery and in the plasma of the umbilical cord obtained by collecting the blood from the cord after delivery.

METHOD

Immediately after withdrawal into a tube containing heparin, blood from the umbilical cord or antecubital vein of pregnant women was centrifuged and the plasma removed. The plasma was extracted with 4 volumes of ether over a thirty-minute period with intermittent shaking. This was repeated twice with 2 volumes for ten minutes each. The ether extracts were combined and shaken with concentrated carbonate solution at pH 10.5, 2*N* alkali, bicarbonate, and then with water much as described by Brown (7). After evaporation of the ether the extract was chromatographed on an alumina column (Harshaw, "catalyst grade") in a manner similar to that described by Eberlein and Bongiovanni (8). The column was first washed with benzene, the extract applied in benzene and then developed with 2 per cent, 4 per cent, and finally with 30 per cent ethanol in benzene. The eluates were evaporated in a vacuum at 60° C. Estriol was found in the 30 per cent ethanol in benzene fractions. Recoveries of estriol from this column averaged 80 per cent. For further purification, partition between toluene and 1*N* sodium hydroxide was performed in the manner described by Engel (9). The estriol so isolated was further purified by paper chromatography in the methylene chloride:formamide system.

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* Presented in part at the 41st Meeting of The Endocrine Society, Atlantic City, New Jersey, June 5, 1959.

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After this procedure the estriol fractions contained nonspecific substances which reacted with ferric chloride-ferricyanide, but remained on the starting line of the paper chromatograms. Thus, it appears that estriol values based on determinations performed on fractions from the column without paper chromatography include the increment due to these nonspecific substances.

RESULTS

The fluorescence spectrum of the isolated estriol dissolved in 88 per cent sulfuric acid was the same as that of authentic estriol; the maximum was found at 480 mμ. The isolated estriol was methylated, and chromatography on alumina carried out as described by Brown (7). The methyl ether so obtained had chromatographic properties on an alumina column similar to those of the reference methyl ether. The fluorescence and excitation spectra of the estriol methyl ether were the same as those of the reference estriol methyl ether. The absorption spectrum of the isolated estriol dissolved in absolute ethanol showed a maximum at 280 mμ. The isolated estriol had a mobility in the methylene chloride:formamide paper chromatogram the same as that of reference estriol. The appropriate areas were eluted from these strips and acetylated in pyridine with acetic anhydride at room temperature overnight to yield an acetate with the same mobility as estriol triacetate in toluene:propylene-glycol paper chromatograms. On paper chromatograms the characteristic green color with ferric chloride-potassium ferricyanide was formed. Although this reaction is not specific for estrogens, many steroids do not react in this manner.

The estriol zone from methylene:chloride chromatograms after elution was subjected to reaction with the p-phenolsulfonic acid-phosphoric acid reagent shown by Bachman (10) to be specific for estriol. This resulted in a violet purple color with maximal absorption at 540 mμ, a reaction which was also shown by authentic estriol.

There was a consistently higher proportion of estriol in umbilical cord blood than in maternal blood. This finding of a greater amount of estriol in umbilical cord blood than in the peripheral maternal venous blood has been reported also in preliminary communications by Aitken and associates (11) and by Roy and Brown (12).

Results of estriol determinations performed on umbilical cord plasma averaged 7.8 μg. per 100 ml., whereas the average level in the maternal peripheral circulation was 4.1 μg. per 100 ml.

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The Endocrine Society

The 1960 Annual Meeting

The Forty-Second Annual Meeting of The Endocrine Society will be held in the Eden Roc Hotel, Miami Beach, Florida, Thursday, Friday and Saturday, *June 9, 10 and 11, 1960.*

The Chairman of Local Arrangements is Dr. Kenneth Savard.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 10, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Eden Roc will hold 250 bedrooms and the adjoining hotels, the Montmartre Hotel and Lucerne Hotel, will hold 75 rooms each for members until May 1, 1960, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 12 for the American Medical Association and other meetings, it is imperative that your reservation be made from June 12 on, directly through the A.M.A. Housing Bureau, c/o Miami Beach Convention Bureau, 1700 Washington Avenue, Miami Beach, Florida, since no reservation beyond June 12 can be accepted directly by the hotels. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1960.

Final program, membership card and advance registration forms will be sent on May 1, 1960 to members whose current dues have been paid.

Program

THURSDAY, JUNE 9, 1960

8:00 a.m.—Registration—Lobby, Eden Roc Hotel

I. 9:00 a.m.—ADRENAL

Cafe Pompeii

Chairman: *Dwight J. Ingle*

1. On the Mechanism by Which Potassium Increases Secretion of Aldosterone.
   Donald S. Gann (by invitation), *Juan F. Cruz* (by invitation), *Alfred G. T. Casper* (by invitation) and *Frederic C. Bartter.*
2. Increased Aldosterone Secretion Following Decapitation and Subsequent Bleeding of Hypophysectomized Dogs.
   James O. Davis, Charles C. J. Carpenter (by invitation), *Carlos R. Ayers* (by invitation) and *Robert C. Bahn.*
3. Aldosterone Secretion Measured During Adrenal Vein Cannulation in Humans.
   Walter H. Moran, Jr. and *Bernard Zimmermann.*
4. Stimulation by Aldosterone of Active Sodium Transport Across a Living Membrane.
   Jean Crabbé.
5. The Inotropic Action of *d,l*-Aldosterone on Isolated Mammalian Cardiac Preparations.
   Ralph D. Tanz (introduced by George Sayers).
6. Effects of Mineralocorticoids on Na^{22} Uptake by Bone.
   David H. P. Streeten, David R. Rovner (by invitation), *Carlos Stevenson* (by invitation) and *Jerome W. Conn.*

INTERMISSION

7. Effects of Chronic Hepatic Venous Congestion on the Disappearance of Tritiated Aldosterone from Plasma.
   Carlos R. Ayers (by invitation), *James O. Davis, Ferol Lieberman* (by invitation) and *Charles C. J. Carpenter* (by invitation).
8. 21-Deoxysteroid Metabolites in Adrenocortical Dysfunction.
   R. I. Cox (introduced by T. F. Gallagher).
9. Studies of Adrenocortical Function of Hirsute Women.
   Arnold M. Moses, Edward L. Klaiber (by invitation), *Julia Lobotsky* (by invitation), *Ross D. Jacobs* and *Charles W. Lloyd.*
10. Studies of Adrenal Nucleic Acids.
    E. D. Bransome, Jr. (by invitation) and *W. J. Reddy.*
11. Some Mechanisms of Steroid Hormone Action.
    Gordon M. Tomkins (invited paper, 20 minutes).

II. 2:00 p.m.—ADRENAL

Cafe Pompeii

Chairman: *Peter H. Forsham*

12. The Effect of Intracarotid Infusions of Cortisol on Impaired Water Diuresis of the Adrenalectomized Dog.

Charles R. Kleeman, Ralph E. Cutler (by invitation) and *Morton H. Maxwell* (by invitation).

13. Maturation of Adrenal Cortical and Medullary Function in the Dog.
B. T. Jackson (by invitation), *J. C. Melby, D. M. Hume* and *R. H. Egdahl.*
14. The Significance of the Concentration of Nonprotein-Bound Plasma Cortisol Concentration in Normal Subjects, Cushing's Syndrome, Pregnancy, and During Estrogen Therapy.
Richard P. Doe, Horace H. Zinneman (by invitation) and *Edmund B. Flink.*
15. Adrenal Secretion of 17α-Hydroxyprogesterone in Normal Dogs.
Balwant D. Agashe (by invitation), *Robert G. Weaver* (by invitation) and *Kristen B. Eik-Nes.*
16. Steroid Studies During and After Prolonged Therapy with Zn-ACTH.
Eleanor Z. Wallace, Linda Leilop (by invitation), *Anne C. Carter* and *Harold A. Lyons* (by invitation).
17. Structure-Function Relationships of Steroids: Dissociation of Classical "Glucocorticoid" Properties by Structural Modificatioh at Carbon-21.
John W. Kendall, Jr. (by invitation) and *Grant W. Liddle.*

INTERMISSION

18. Corticosteroids and Accumulation of Substrate by Isolated Rat Diaphragm: Possible Mechanism of Anti-Inflammatory Action.
Ira G. Wool.
19. The *in Vitro* Reduction of 11β-Hydroxy and 11-Ketosteroids by Liver Δ^4-Hydrogenases.
Enrico Forchielli (by invitation), *Keith Brown-Grant* (by invitation) and *Ralph I. Dorfman.*
20. Experimental Immunologic Adrenal Injury.
Jan Steiner (by invitation), *Douglas L. Schatz* (by invitation), *Bernard Langer* (by invitation) and *Robert Volpé.*
21. Effect of Hypercapnia on Plasma Epinephrine and Norepinephrine Concentration.
J. C. Rosenberg (by invitation), *B. Goott* (by invitation), *R. C. Lillehei* (by invitation), *F. Miller* (by invitation) and *Bernard Zimmermann.*
22. Inhibition of Pituitary ACTH Secretion as the Major Glucocorticoid Effect of a Synthetic Steroid.
Donald A. Holub, Fred H. Katz (by invitation) and *Joseph W. Jailer.*
23. Hormonal Influence on RNA Synthesis in the Liver.
K. E. Paschkis, A. Cantarow and *T. H. Williams* (by invitation).

III. 2:00 p.m.—PITUITARY

Mona Lisa Room

Chairmen: *George Sayers* and *William H. Daughaday*

24. Further Purification Studies on Corticotropin Releasing Factor (CRF).
Andrew V. Schally, R. N. Andersen (by invitation), *Harry S. Lipscomb, John M. Long* (by invitation) and *Roger Guillemin.*
25. Apparent Dissociation Between Adrenal and Extra-Adrenal Actions of Corticotropin Chemically Altered by Periodate-Borohydride Treatment.
H. Angus Boright (by invitation), *Frank L. Engel, Jack L. Kostyo* and *J. Earle White, Jr.*

26. Modification of Pituitary-Adrenal Response to SU-4885 by Psychotropic Drugs.
Ernest M. Gold (by invitation), *John R. Kent* (by invitation) and *Peter H. Forsham.*
27. Studies on Adrenal Cortical and Anterior Pituitary Responsiveness.
T. Albert Farmer, Jr. (by invitation), *J. W. Herod, Jr.*, (by invitation). *James A. Pittman, Jr.* and *S. Richardson Hill, Jr.*
28. The Estimation of ACTH in Human Plasma.
V. K. Vance (by invitation), *W. J. Reddy, D. H. Nelson* and *G. W. Thorn.*
29. Disturbances of the Servo-Mechanism Regulating Adrenal Steroidogenesis in Patients with Cerebral Disease.
Jack H. Oppenheimer (by invitation), *Leonard V. Fisher* (by invitation) and *Joseph W. Jailer.*

INTERMISSION

30. Estimates of the Secretion Rate of Thyrotropin in the Normal and Hypothyroid Rat and Man.
J. L. Bakke and *N. Lawrence* (by invitation).
31. Extrathyroidal Actions of Pituitary Thyrotropin *in Vitro*: Effects Upon Rat Adipose Tissue.
Norbert Freinkel.
32. Effect of Dietary Protein Deprivation on Pituitary Tropic Hormones in Normal and Acromegalic Subjects.
R. Palmer Howard and *Robert H. Furman.*
33. Neuroendocrinological Syndrome of Galactorrhea, Papilledema and Increased Intracranial Pressure.
Joseph F. Dingman, Eduardo Gaitan (by invitation), *Akira Arimura, Max C. Staub* (by invitation) and *Ruth Paterson* (by invitation).
34. Glass Paper Chromatographic Method for Vasopressin and Oxytocin in Human Plasma.
Akira Arimura and *Joseph F. Dingman.*
35. An Antidiuretic Effect of Chlorothiazide in Patients with Diabetes Insipidus.
Richard M. Portwood, Walter C. Lusk, and *E. C. Buckley* (introduced by *Frank L. Engel*).

IV. 7:30 p.m., Thursday, June 9—TESTIMONIAL DINNER FOR ROY HOSKINS—Imperial Room

FRIDAY, JUNE 10, 1960

8:30 a.m.—Registration—Lobby, Eden Roc Hotel

V. 9:00 a.m.—REPRODUCTION. CALCIUM METABOLISM

Cafe Pompeii

Chairmen: *Roy Hertz* and *Gilbert S. Gordan*

36. Ultrastructure of the Placenta.
Edward W. Dempsey (invited paper, 20 minutes).
37. 6β-Hydroxy-Cortisol: Abnormally High Levels in Human Urine in Pregnancy and Toxemia.
Fred H. Katz (by invitation), *Andrew G. Frantz* (by invitation) and *Joseph W. Jailer.*

38. Progesterone Levels in the Rabbit Ovarian Vein Blood Throughout Pregnancy.
Girgis Mikhail (by invitation), *Matthew W. Noall* (by invitation) and *Willard M. Allen.*
39. The Nature of Uterine Phenol-Activated DPNH Oxidase.
Vincent P. Hollander and *Jean R. Beard* (by invitation).
40. Inhibition of Glucuronosyl Transferase by Steroid Hormones.
Robert M. Dowben and *David Y. Y. Hsia* (by invitation).

INTERMISSION

41. The Use of Norethynodrel in Fertility Control.
Edward T. Tyler.
42. New Criteria of "Target" Tissues for Steroid Estrogens.
Elwood V. Jensen
43. Inhibitory Effects of Hypercalcemia on Secretion of Parathyroid Hormone.
Laurence H. Kyle, John J. Canary, Daniel H. Mintz (by invitation) and *Antonio C. de Leon* (by invitation).
44. Hypercalciuria Associated with "Steroid Withdrawal" in Cushing's Syndrome.
Samuel H. Bassett and *Stewart G. Tuttle* (by invitation).
45. Unifying Concepts of Parathyroid Hormone Action.
A. Raymond Terepka, Charles Dowse and *William F. Neuman* (invited paper, 20 minutes).

1:30 p.m.—ANNUAL BUSINESS MEETING

Cafe Pompeii

VI. 2:30 p.m.—PITUITARY. TESTES

Cafe Pompeii

Chairmen: *Charles W. Lloyd* and *Warren O. Nelson*

46. Effects of Human Growth Hormone and its Subfractions on Sodium Retention.
Edward G. Biglieri (by invitation), *Charles O. Watlington* (by invitation) and *Vincent C. Di Raimondo.*
47. Hormonal Activity of Choriocarcinoma in Women and in Heterologous Transplants in Hamsters and Rats.
Roy Hertz, M. B. Lipsett and *S. J. Jackson* (by invitation).
48. Immunological Analysis of Ovine Pituitary LH.
Sheldon J. Segal.
49. The Action of Gonadotropic Hormone Preparation from Various Species in the Hypophysectomized Male Rhesus Monkey,
E. Knobil and *J. B. Josimovich* (by invitation).
50. The Maintenance of Luteotropin Secretion by Means of Progesterone.
I. Rothchild.
51. Urinary Excretion of Luteinizing Hormone (LH) by Anxious Patients and Normal Subjects.
Harold Persky, Howard C. Jackson (by invitation), *Robert W. McCurdy* (by invitation) and *John Paul Brady* (by invitation).

INTERMISSION

52. OBSERVATIONS ON THE ENDOGENOUS RESPIRATION OF THE TESTICULAR TISSUE.
E. Steinberger and *C. Wagner* (by invitation).
53. THE DISTRIBUTION OF TESTOSTERONE-4-C^{14} IN THE RAT.
F. Parra (by invitation), *W. J. Reddy* and *G. W. Thorn*.
54. EFFECTS OF ANDROGENS ON INTERMEDIARY METABOLISM IN MUSCLE.
C. A. Villee, J. M. Loring (by invitation) and *J. M. Spencer* (by invitation).
55. *In Vivo* GLYCINE-1-C^{14} INCORPORATION STUDIES OF MOUSE KIDNEY β-GLUCURONIDASE IN RESPONSE TO ANDROGENIC STIMULATION.
Olive S. Pettengill (by invitation) and *William H. Fishman*.
56. STIMULATION OF ERYTHROPOIESIS BY ANDROGENIC HORMONES.
B. J. Kennedy.
57. THE TESTIS IN MYOTONIC DYSTROPHY (STEINERT'S DISEASE).
Nicholas P. Christy, William D. Drucker (by invitation) and *William A. Blane*.

VII. 2:30 p.m.—OVARY. THYROID

Mona Lisa Room

Chairmen: *E. B. Astwood* and *S. H. Ingbar*

58. COMPARATIVE HYDROLYSIS OF ESTROGEN CONJUGATES BY THREE DIFFERENT ENZYME PREPARATIONS.
Avery A. Sandberg and *W. R. Slaunwhite, Jr.*
59. A STUDY OF ESTROGEN ANTAGONISM BY MER-25.
Arthur Cutler (by invitation), *Jeanne A. Epstein* and *Herbert S. Kupperman*.
60. DISCREPANCIES BETWEEN VARIOUS I^{131} PREPARATIONS IN MEASUREMENT OF THYROID I^{131} UPTAKES.
Alvin Taurog.
61. THE *in Vitro* STIMULATION OF THYROIDAL ACTIVITY BY PROPYLTHIOURACIL.
Philip F. Mulvey, Jr. (by invitation), *D. Ward Slingerland* (by invitation) (introduced by Belton A. Burrows).
62. THE THYROIDAL ORGANIFICATION OF RADIOIODINE IN THE PRESENCE OF INHIBITORY AMOUNTS OF ANTITHYROID SUBSTANCE.
Marvin L. Mitchell, Jose A. Sanchez-Martin (by invitation), *Anne B. Harden* (by invitation) *and Mary E. O'Rourke* (by invitation).
63. EFFECT OF THYROTROPIN UPON THYROIDAL IODIDE RELEASE IN MAN AND RAT.
I. N. Rosenberg, C. S. Ahn (by invitation) and *M. H. Chalfen* (by invitation).

INTERMISSION

64. EFFECTS OF SEROTONIN AND RELATED COMPOUNDS ON THE METABOLISM OF THYROID HORMONES AND IODINE.
Valerie Anne Galton (by invitation) and *Sidney H. Ingbar*.
65. THE ROLE OF GLUCOSE AND INSULIN IN PERIPHERAL THYROXINE DEGRADATION AND THYROIDAL IODIDE ACCUMULATION.
Herbert G. Rose (introduced by John R. Hogness).
66. THE INTERACTION OF SERUM THYROXINE DURING PATHOLOGIC PREGNANCIES WITH ION EXCHANGE RESINS.
R. L. Nicoloff (by invitation), *J. T. Nicoloff* (by invitation) and *J. T. Dowling*.
67. INHERITANCE OF DEFECTIVE ORGANIC BINDING IN FAMILIAL GOITROUS CRETINISM.
Rose H. Parker (by invitation) and *William H. Beierwaltes*.

68. Effect of Thyroid Deficiency on Amino Acid Transport in the Adenohypophysis.
Paulo A. Ayroza-Galvao (by invitation), *David M. Kipnis* and *William H. Daughaday.*

69. Dissociation of Calorigenic and Cholesterol Lowering Effects by Diiodothyroacetic Acid.
John R. Kent (by invitation), *Harry F. Hurd* (by invitation), *Edwin S. Stenberg* (by invitation) and *Peter H. Forsham.*

VIII. ANNUAL DINNER—Eden Roc Hotel

Dwight J. Ingle, President, presiding

6:30 p.m. Cocktails—Pavilion Room.
Sponsored by the donors of the Awards of the Society:
Ciba, Ayerst, Upjohn and Squibb. Attendance limited to holders of dinner tickets.

7:30 p.m. Dinner—Imperial Room.
Presentation of the 1960 Awards of the Society by *Roland K. Meyer*:
The Fred Conrad Koch Award
The Ciba Award
Squibb Fellowship
Upjohn Scholar
President's Address: *Dr. Ingle.*

SATURDAY, JUNE 11, 1960

8:30 a.m.—Registration—Lobby, Eden Roc Hotel

IX. 9:00 a.m.—DIABETES AND RELATED METABOLISM

Cafe Pompeii

Chairmen: *Thaddeus S. Danowski* and *Robert H. Williams*

70. The Diagnostic Value and Modus Operandi of Sodium Tolbutamide in Hypoglycemic States.
Stefan S. Fajans, Jack M. Schneider (by invitation), *David E. Schteingart* (by invitation) and *Jerome W. Conn.*

71. Transhepatic Balance of Insulin-Like Activity Following Hyperglycemia.
R. H. Egdahl and *H. L. Goldberg* (by invitation).

72. Studies on the Binding of Insulin to Adipose Tissue *in Vitro.*
Rene Humbel (by invitation) and *Albert E. Renold.*

73. Effect of Plasma Protein Fractions from Normal and Hypophysectomized Subjects on Glucose Uptake by Muscle.
K. W. Taylor (introduced by A. E. Renold).

74. Interference by Epinephrine, ACTH and Growth Hormone in Measurements of Insulin-Like Activity (ILA).
Jurgen Steinke (by invitation), *Gaston Zahnd* (by invitation) and *Albert E. Renold.*

75. Hormonal Effects on Pathways of Glucose Metabolism of Endocrine Tissues *in Vitro.*
Ira Pastan, James B. Field and *Arnold N. Weinberg.*

INTERMISSION

76. Effects of Human, Monkey, Pig, and Sheep Growth Hormones on Glucose Metabolism in Adipose Tissue.
Albert I. Winegrad and *Walter N. Shaw* (introduced by Francis D. W. Lukens).
77. The Lipolytic Response to Corticotropin.
C. H. Hollenberg (by invitation), *M. S. Raben* and *E. B. Astwood.*
78. An *in Vitro* Effect of Cortisol on ACTH or Epinephrine Stimulated Adipose Tissue.
Bernard Leboeuf (by invitation) and *George F. Cahill, Jr.*
79. Influence of Hypophysectomy and Certain Hormones on a-Aminoisobutyrate (AIB) Transport in Adrenal and Diaphragm.
J. Eichhorn (by invitation), *I. D. K. Halkerston* (by invitation), *M. Feinstein* (by invitation) and *O. Hechter.*
80. Experience with Melatonin, A Pigment Cell Hormone from the Pineal.
James D. Case (by invitation), *Aaron B. Lerner* (by invitation), *M. Ruth Wright* (by invitation) and *Joseph S. McGuire* (by invitation). (Invited paper, 20 minutes.)

X. 2:00 p.m.—CLINICAL SESSIONS

Cafe Pompeii

Chairman: *John E. Howard*

1. Control of Ovulation.
Charles W. Lloyd, Robert B. Greenblatt and *Gregory Pincus.*
2. Abnormalities of Sex Differentiation.
   A. Classification and Etiology.
   Melvin M. Grumbach.
   B. Diagnosis and Selection of Gender of Rearing.
   Lawson Wilkins.
   C. Psychologic Aspects of Sex.
   John Money.
   D. Questions and Presentation of Cases.
3. Tests of Adrenocortical Function.
   J. W. Jailer, L. J. Soffer, N. P. Christy and R. E. Peterson.

The 1960 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J. B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

The Ciba Award

The Ciba Award, to recognize the meritorious accomplishments of an investigator in the field of clinical or preclinical endocrinology who has not attained his thirty-sixth birthday by June 1 of the year in which the award is presented, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer; 1959—Dr. Gordon L. Farrell. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional

promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

Twelfth Postgraduate Assembly in Endocrinology and Metabolism

UNDER THE CO-SPONSORSHIP OF THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

MADISON, WISCONSIN

October 24 through 28, 1960

For further information, please write to the local chairman, Dr. Edgar S. Gordon, The University Hospitals, 1300 University Avenue, Madison 6, Wisconsin. The fee is $100.00 for physicians and $30.00 for Residents or Fellows taking the course.

First International Congress of Endocrinology

The First International Congress of Endocrinology will be held in *Copenhagen, Denmark, July 18–23, 1960.*

United States members of the International Executive Committee are Dr. Warren O. Nelson and Dr. Leo T. Samuels, Dr. Gregory Pincus is Chairman of the Program Committee. The tentative program consists of ten symposia covering the general field of endocrinology plus round-table discussions and a number of submitted papers. Those desiring to submit papers should immediately contact Dr. Gregory Pincus, Worcester Foundation, Shrewsbury, Massachusetts. There will be a number of interesting scientific and technical exhibits.

In order to facilitate travel arrangements to the Copenhagen Convention, the Council of The Endocrine Society at its San Francisco meeting designated THOMAS COOK & SON as our authorized travel agents. They will in turn offer especially designed All Expense Paid Tours to Europe, including hotel reservations for the convention, as well as offering independent itineraries for any of our members who desire to travel separately by air or by steamship.

Registration is open to anyone interested in endocrinology at an approximate fee of $28 U.S. Members of the families of those attending may register as associates at an approximate fee of $14 U.S., fee to be paid at the time of registration or in advance to the American Express Company, which has been designated by the Executive Committee of the International Congress as official financial and travel agent. There will be a number of social functions and other entertainment provided for all registrants.

As you all know, European travel is unusually heavy during July and it is suggested that those contemplating attending the Congress should start making tentative reservations at the earliest possible moment by filling in and returning the card recently mailed to members, to THOMAS COOK

& SON's special representative—Harry E. Kornbaum, Transportation Chairman, Rainbow Travel Service, 2817 Classen Boulevard, Oklahoma City 6, Oklahoma, who will correspond directly with each member about his individual travel arrangements to and from the Convention, at the same time keeping our National Officers fully informed of all travel arrangements of its members. Completion of the card CARRIES NO OBLIGATION OR COMMITMENT ON YOUR PART. Send it in today for the complete travel arrangements for our Society.

The general expenses of a meeting of this magnitude are quite large and The Endocrine Society has made a substantial contribution from its treasury for organizational purposes. U. S. pharmaceutical firms have also contributed very generously. However, sufficient funds are not as yet available to assure the success of this meeting; therefore, *the committee suggests that each of our members make voluntary contributions to this fund.* Please make your checks payable to: The Endocrine Society—First International Congress of Endocrinology. Mark it *Contribution* and mail it to the office of the Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

Program

The program of the First International Congress of Endocrinology which will be held in Copenhagen, Denmark, July 18–23, 1960, will consist of ten symposia and a round table discussion, along with sessions of communicated papers. The speakers at the symposia and the round table discussion have been invited by the Subcommittee on Program of the Congress. In Symposia #1–8 and in #10, the Subcommittee has also invited a discusser for each principal paper. Symposium #9 will consist of six principal papers and no discussers. The round table discussion will consist of eight sections, each with a principal speaker and three secondary speakers.

(For program see the December 1959 issue of the Journal.)

The American Goiter Association

1960 Annual Meeting and the 4th International Goiter Conference

The next Annual Meeting of the American Goiter Association will be held in *London, England, July 5–9, 1960,* at Church House, Dean's Yard, Westminster, London, S.W.1. This meeting, co-sponsored with the London Thyroid Club, will be the Fourth International Goiter Conference. For further information write Dr. Selwyn Taylor, Royal Society of Medicine, Dering Yard, 67c New Bond Street, London, W.1, England.

JOHN C. MCCLINTOCK, *Secretary,*
149½ Washington Avenue,
Albany 10, N.Y.

Postgraduate Course in the Clinical Uses of Radioiodine

An intensive didactic, laboratory and clinical course in the diagnostic and therapeutic uses of radioiodine. Faculty for the course is the staff of the Department of Radiology of the University of Texas Southwestern Medical School. This year's Visiting Lecturer will be Reginald A. Shipley, M.D., Chief of the Radioisotope Unit, U. S. Veterans Administration Hospital and Department of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio. The course is intended to qualify the enrollee for Atomic Energy Commission licensure. Enrollment is limited to ten in a course to be conducted from *June 20 through June 25, 1960.* Additional information may be obtained from Frederick J. Bonte, M.D., Professor and Chairman, Department of Radiology or John S. Chapman, M.D., Assistant Dean, University of Texas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, Texas.

Inter-Society Cytology Council

The Inter-Society Cytology Council, Annual Scientific Meeting will be held in the Palmer House, Chicago, Illinois, *September 23–25, 1960.* Paul A. Younge, M.D., Secretary, 1101 Beacon Street, Brookline 46, Massachusetts.

Sixth International Congress of Internal Medicine

The Sixth International Congress of Internal Medicine will be held *August 24–27, 1960 in Basle, Switzerland.* For program and registration forms, write (before April 30, if possible) to the Secretary, Steinentorstrasse 13, Basle 10, Switzerland.

Book Register

(*The*) *Central Nervous System and Behavior. Translations from the Russian Medical Literature.* Collected for the Participants of the Third Macy Conference on the Central Nervous System and Behavior held under the Joint Sponsorship of The Josiah Macy, Jr. Foundation and the National Science Foundation. The volume contains 70 articles on various research aspects of neurology and psychiatry in children and adults. As stated by DR. FRANK FREMONT-SMITH, Medical Director, Josiah Macy, Jr. Foundation, in the Preface: "Many of the articles in this volume are not normally available to American scientists who do not read the Russian language. Some have appeared, however, in journals translated for the National Institutes of Health. The articles selected do not necessarily represent the best research conducted in Soviet laboratories, but were selected as being generally representative of some of the more recent Soviet research concerned with the understanding of human behavior. The Russian work has interest for two reasons: first, because it represents a sample of more development of a long-standing historical interest in the central nervous system; and second, because communication between Soviet scientists and scientists of the Western world has been inadequate"; 1062 pages; 1959. U. S. Department of Health, Education, and Welfare, Public Health Service. Distributed to medical libraries throughout the United States and Canada by the Russian Scientific Translation Program, National Institutes of Health, Bethesda, Maryland.

Ciba Foundation Study Group

No. 3 *Cancer of the Cervix: Diagnosis of Early Forms.* In honor of PROF. DR. C. KAUFMANN, Universitäts-Frauenklinik, Cologne, Germany. Edited by G. E. W. WOLSTENHOLME and M. O'CONNOR for the Ciba Foundation. Twenty-three participants discussed the following subjects: Definition and Classification of the So-called Carcinoma *in Situ;* Histopathological Problems Concerning the Early Diagnosis of Carcinoma of the Cervix; Chromosomal and Cytoplasmic Cytopathology of Intra-epithelial Squamous-Cell Epithelioma of the Cervix Uteri; Vaginal Fluid β-Glucuronidase in Relation to Cancer of the Cervix; The Morphological Changes in the Cervix Uteri with Age; The Problems of Detection of Carcinoma of the Cervix and Evaluation in Regard to Therapy; Atypical Hyperplasia of the Endometrium, with an Attempted Definition of Stage O Carcinoma Corporis Uteri; 114 pages; illustrated; 1959. Boston, Massachusetts; Little, Brown and Company. Price $2.50.

Detoxication Mechanisms. The Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds, second edition. By R. TECWYN WILLIAMS, Ph.D., D.Sc., Professor of Biochemistry in the University of London at St. Mary's Hospital Medical School. Since this book was first published in 1947,

the advances in this field have been so great that the book has been completely rewritten and enlarged about two and a half times. There are chapters on the historical aspects and on the metabolism of the following: simpler aliphatic compounds; aliphatic alcohols, glycols and polyols; metabolism of some aliphatic aldehydes, ketones and acids; metabolism of *cyclo*hexane and its derivatives; metabolism of aliphatic amines and amides; metabolism of aromatic hydrocarbons, halogenated aromatic hydrocarbons; phenols; aromatic alcohols, ethers, aldehydes, ketones and quinones; aromatic acids; nitriles; aromatic nitro compounds; aromatic amines; dyestuffs and other coloring matters; sulphones, sulphonic acids and sulphonamides; terpenes and camphors; heterocyclic compounds, monocyclic and polycyclic; organic compounds of phosphorus and arsenic and certain organo-metallic compounds; foreign compounds and the detoxication mechanisms; 796 pages; formulae and tables; 1959. John Wiley & Sons, Inc., New York 16, N.Y. Price $19.00.

Diabetes: With a Chapter on Hypoglycemia. By 54 Authors; edited by ROBERT H. WILLIAMS, M.D., Executive Officer and Professor of Medicine, University of Washington; Physician-in-Chief, University Hospital, Seattle, Washington. A condensation of the most authoritative, up-to-date information on the research and clinical aspects of diabetes mellitus. The authors were selected on the basis of their research contributions and also for their effectiveness as teachers and writers. As stated in the Preface, descriptions are presented of the chemistry of insulin and its distribution and fate in the body, as well as its role in carbohydrate, fat and protein metabolism. There is consideration of allied actions of other hormones, particularly glucagon, epinephrine, corticosteroids and growth hormone, and the effect of these hormones on insulin responsiveness and stabilization of carbohydrate metabolism. "Factors are described in the serum which are antagonistic to insulin, accounting for marked insulin resistance. Many aspects of the etiology, pathogenesis, pathology, clinical manifestations, and diagnosis of diabetes are discussed. There is extensive consideration of the clinical management of diabetes. Meticulous attention is given to the most recent investigations dealing with tolbutamide, chlorpropamide, metahexamide, phenethylbiguanide and other oral antidiabetic compounds. Several chapters are devoted to major concomitants of diabetes and to different types of diabetes. The special problems of diabetes in children and during pregnancy are discussed. Extensive attention is given to the problem of hypoglycemia." New tests for prediabetes and early diabetes are included; 794 pages; 192 illustrations, 23 in full color; 1960. Paul B. Hoeber, Inc., New York 16, N.Y. Price $20.00.